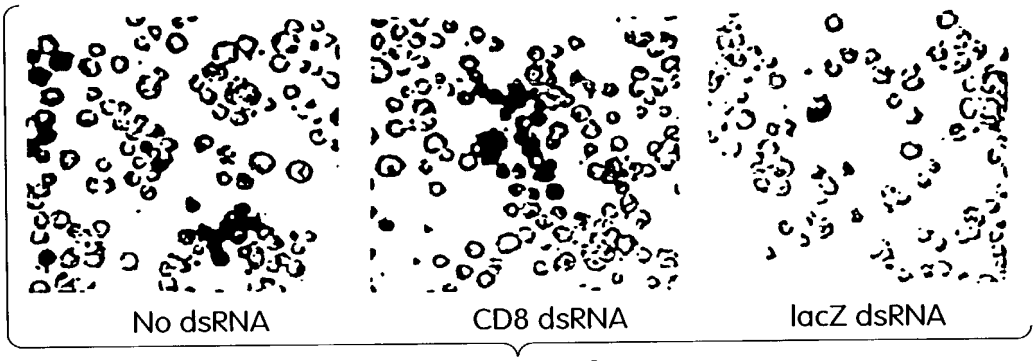


Fig. 1A

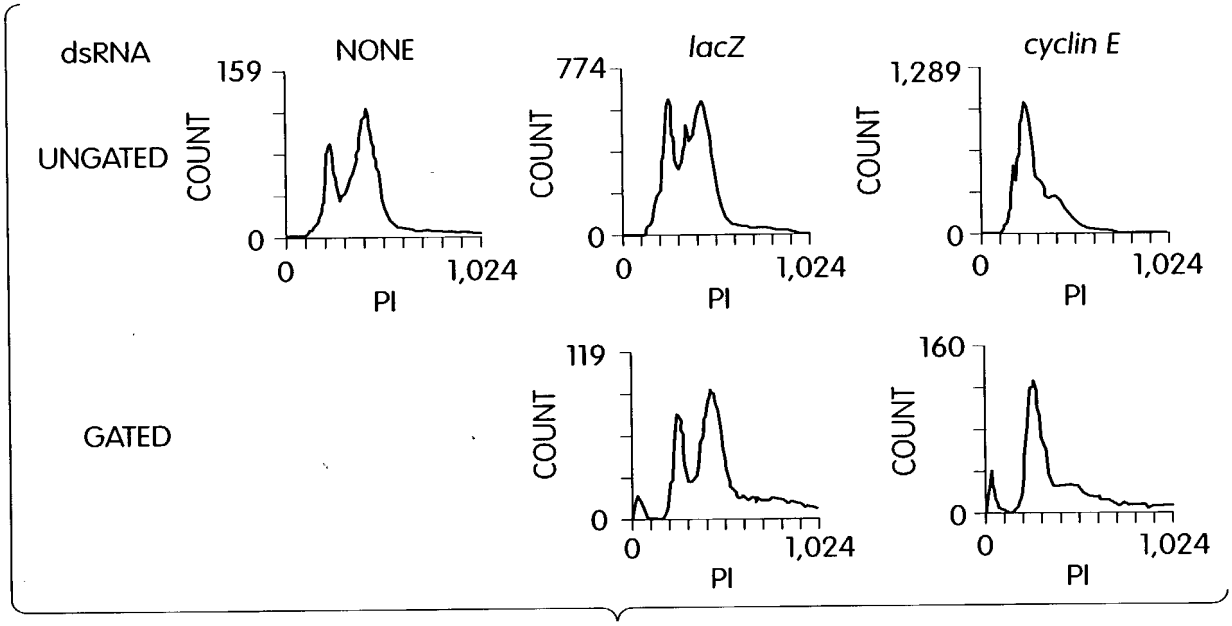


Fig. 1B

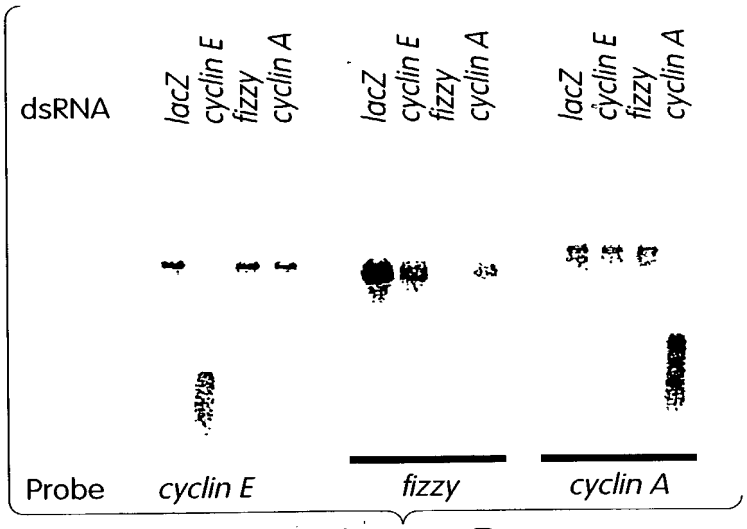


Fig. 1C

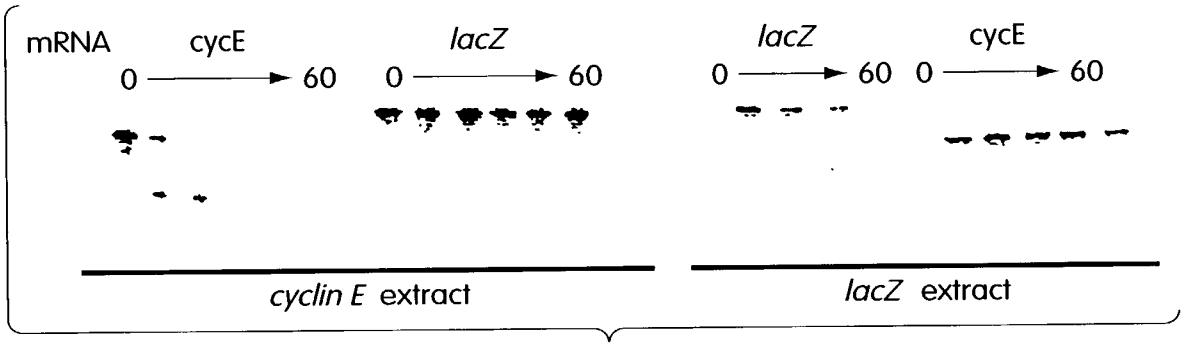


Fig. 2A

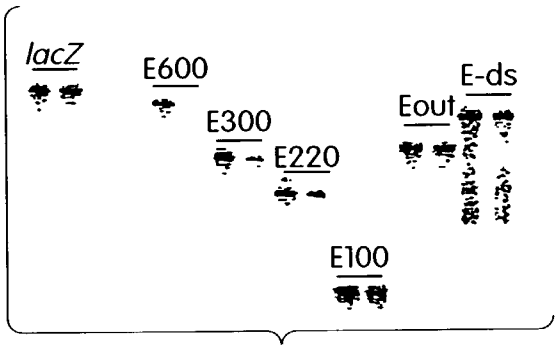


Fig. 2B

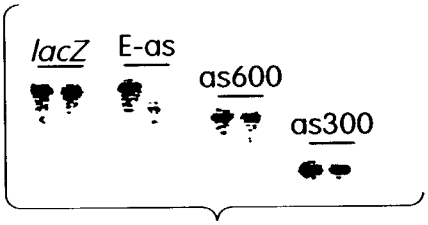


Fig. 2C

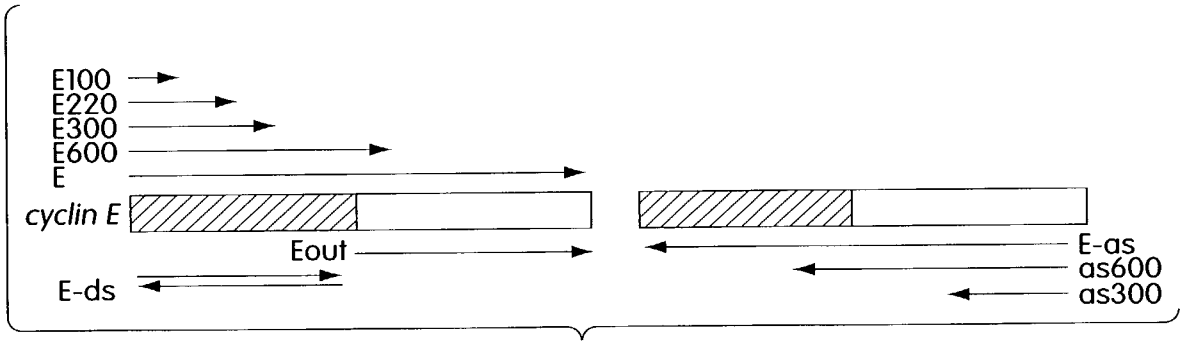


Fig. 2D

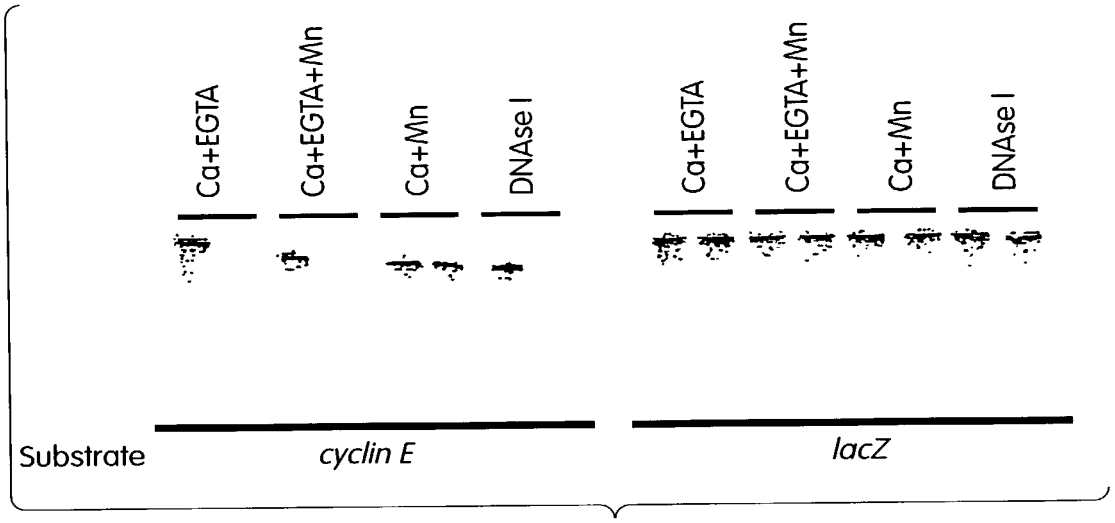


Fig. 3

Fig. 4A

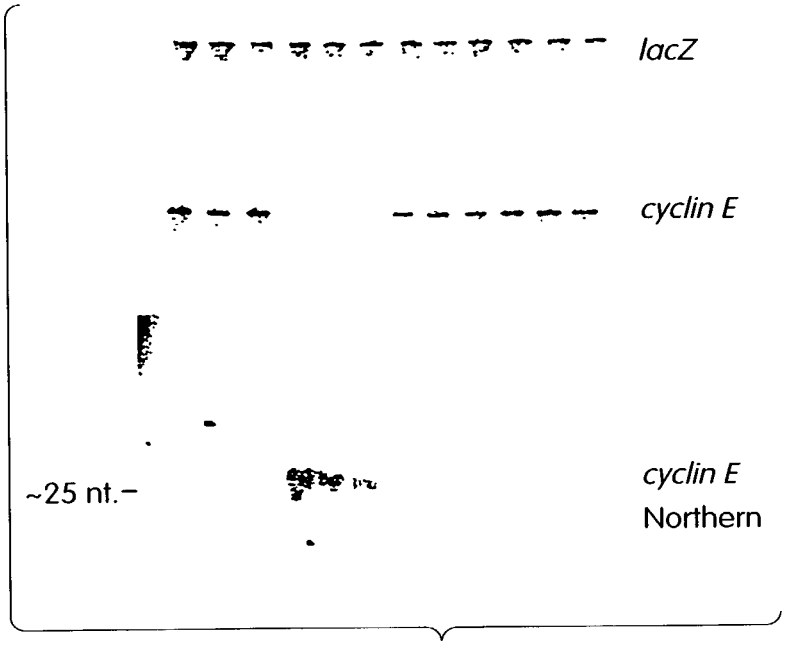
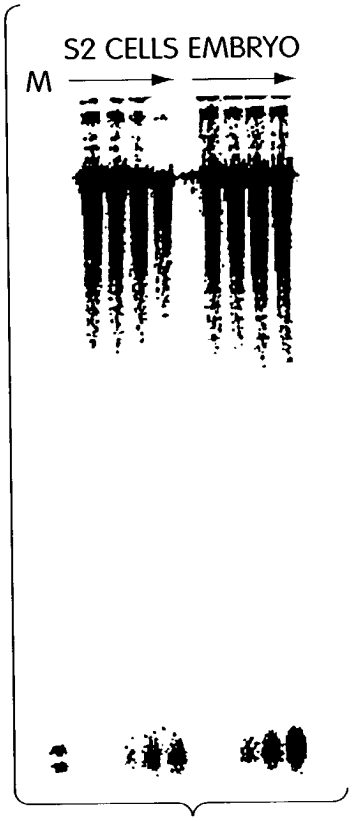


Fig. 4B



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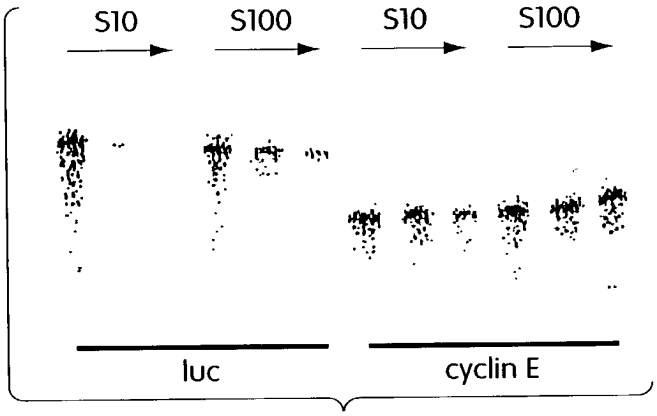

Fig. 5B

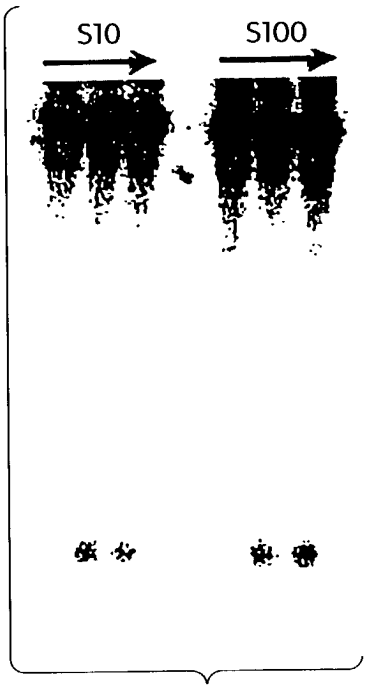

Fig. 5C

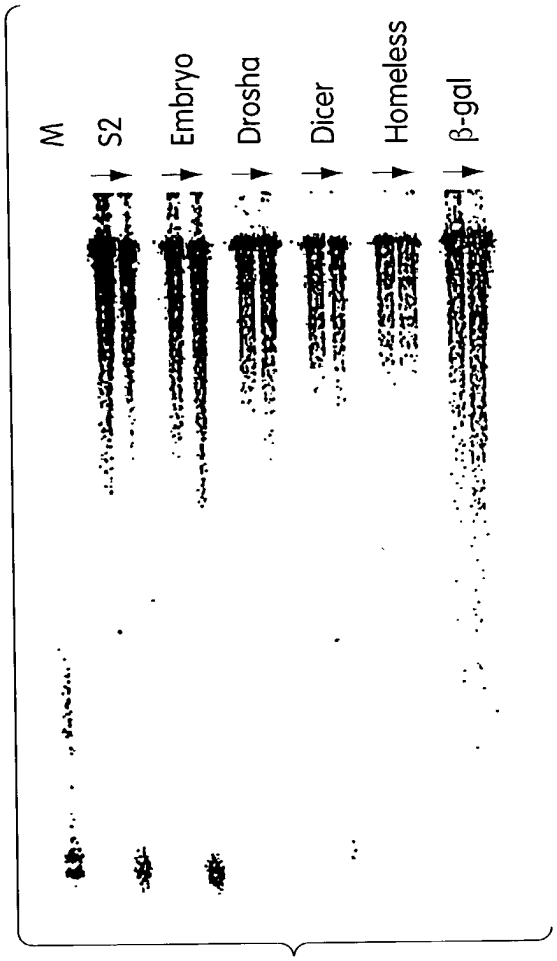

Fig. 6A

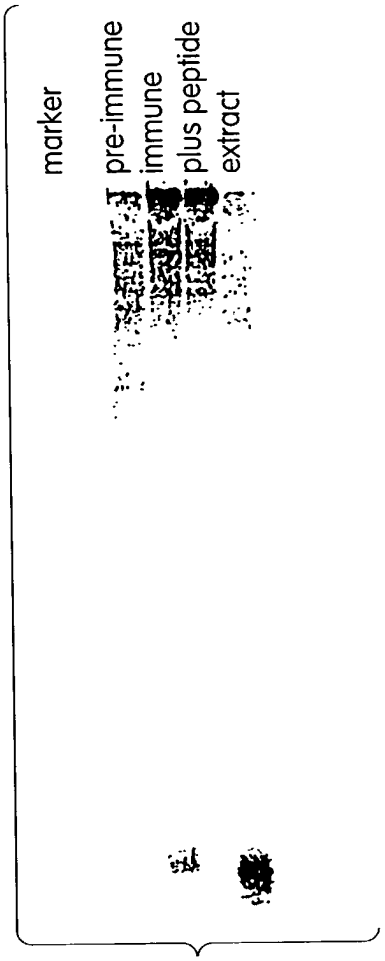

Fig. 6C

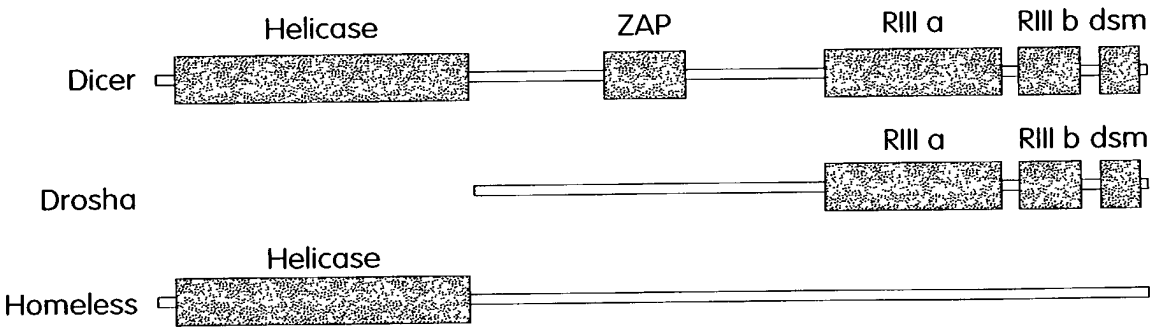

Fig. 6B

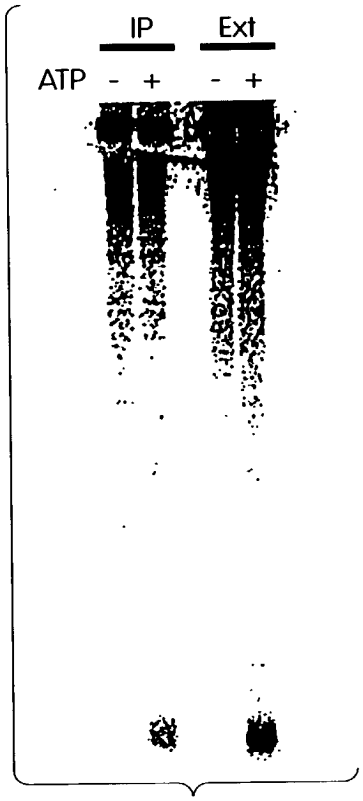

Fig. 6D

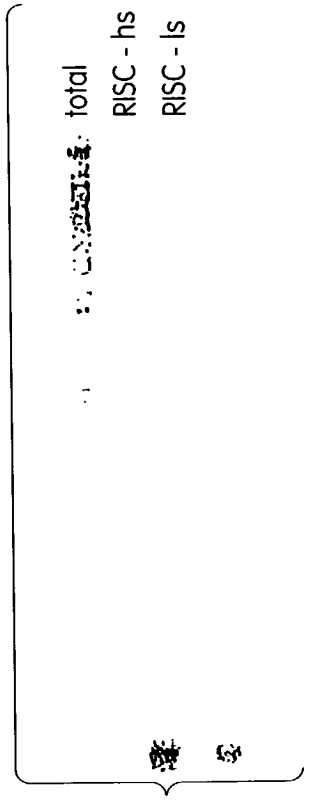

Fig. 6E

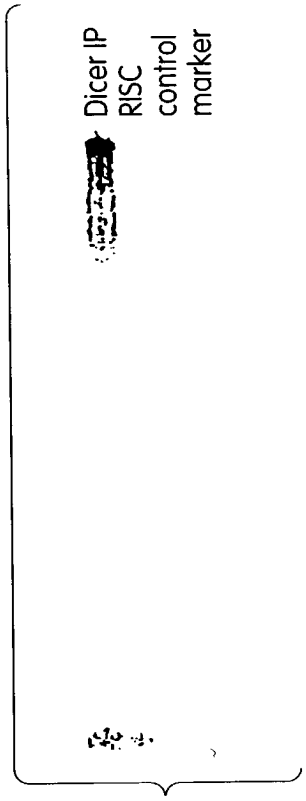

Fig. 6F

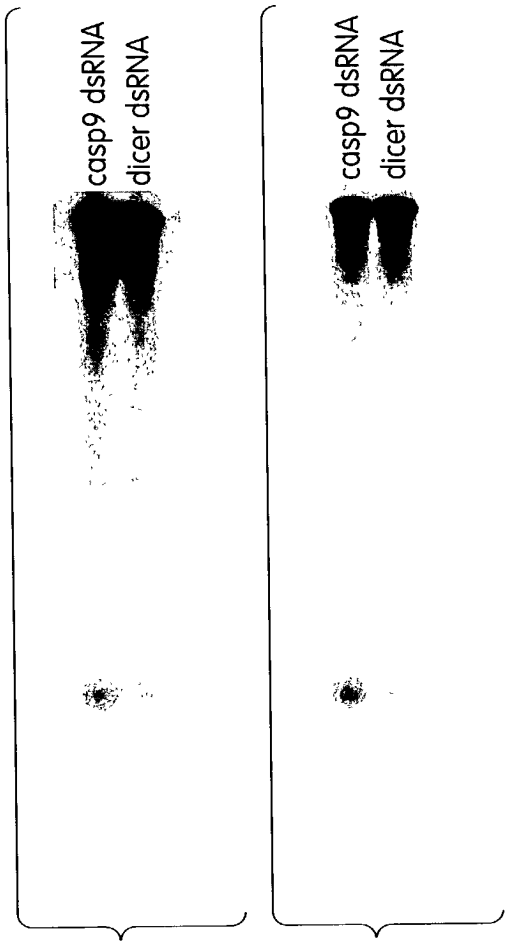

Fig. 7A Fig. 7B

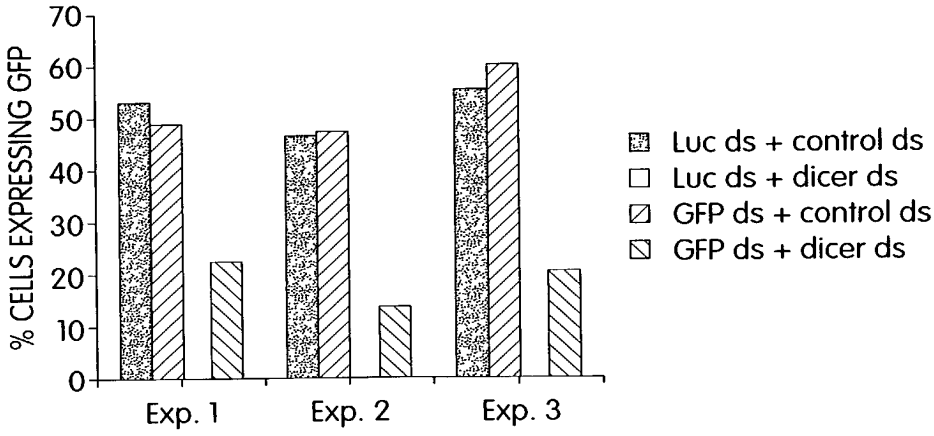

Fig. 7C

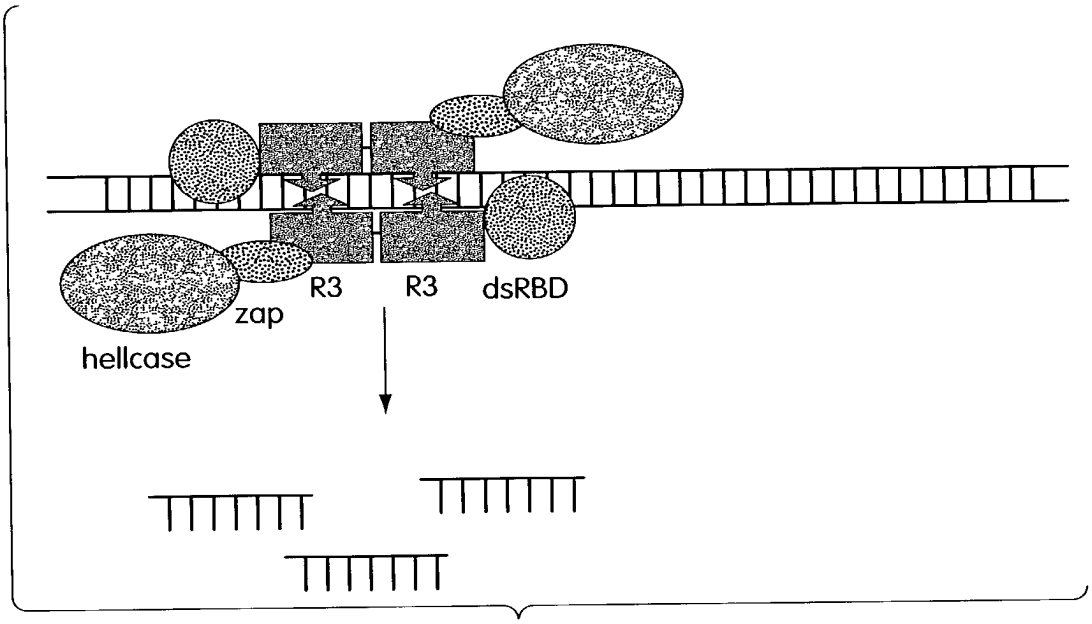

Fig. 8A

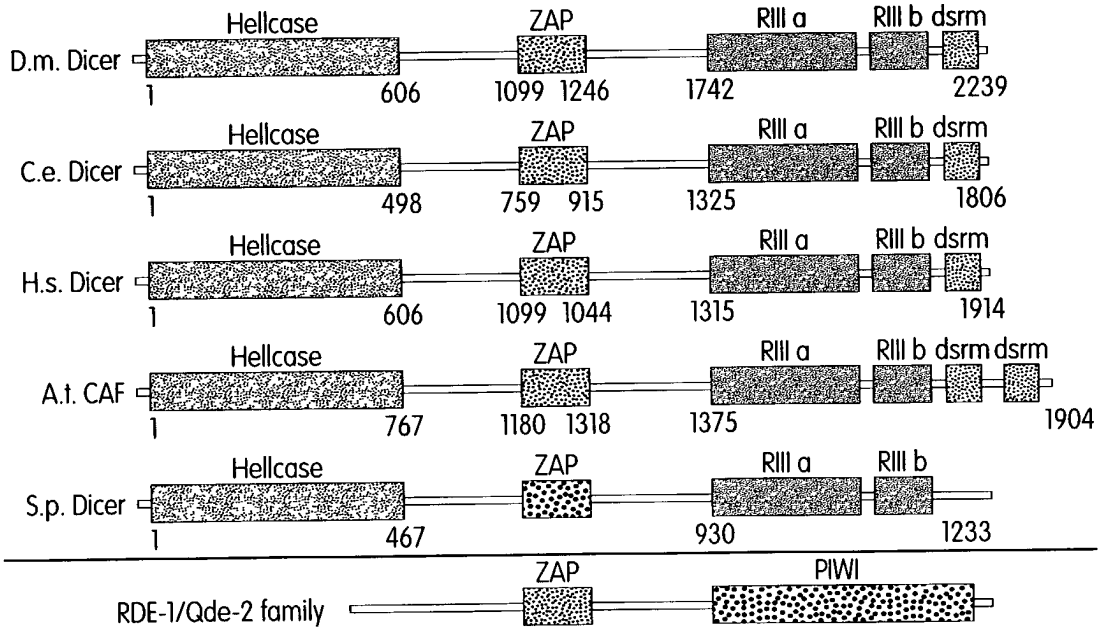

Fig. 8B

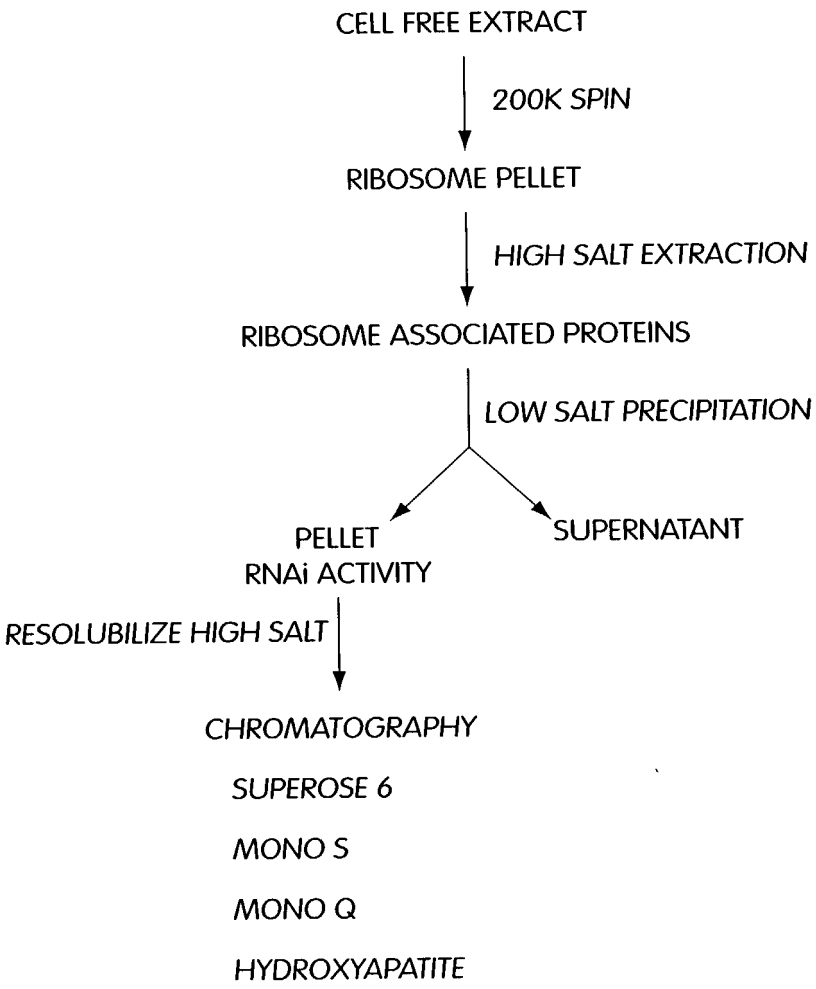

Fig. 9

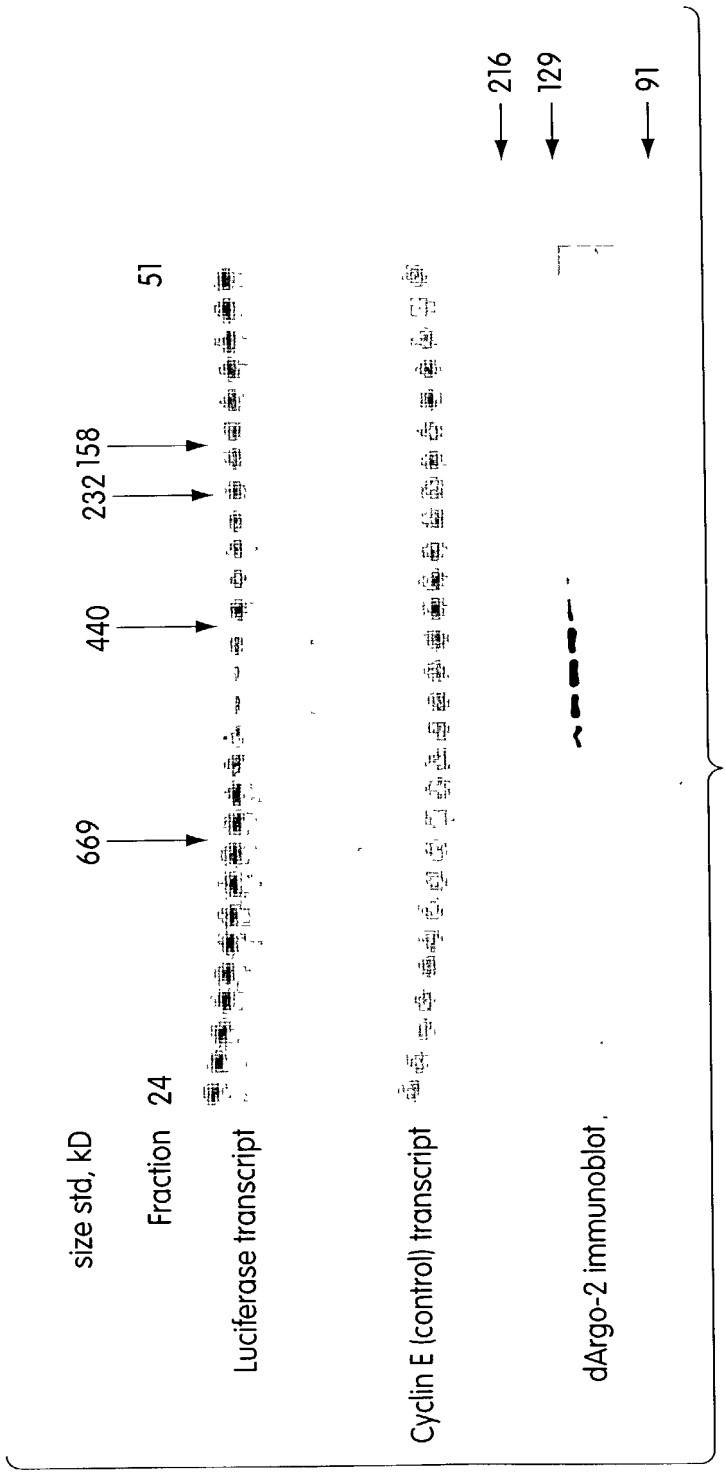

Fig. 10

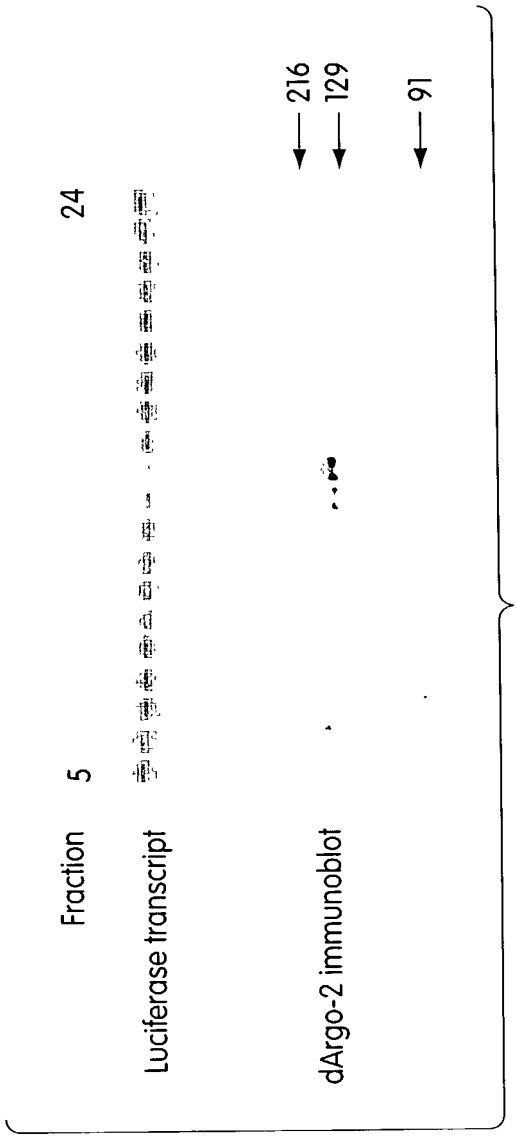

Fig. 11

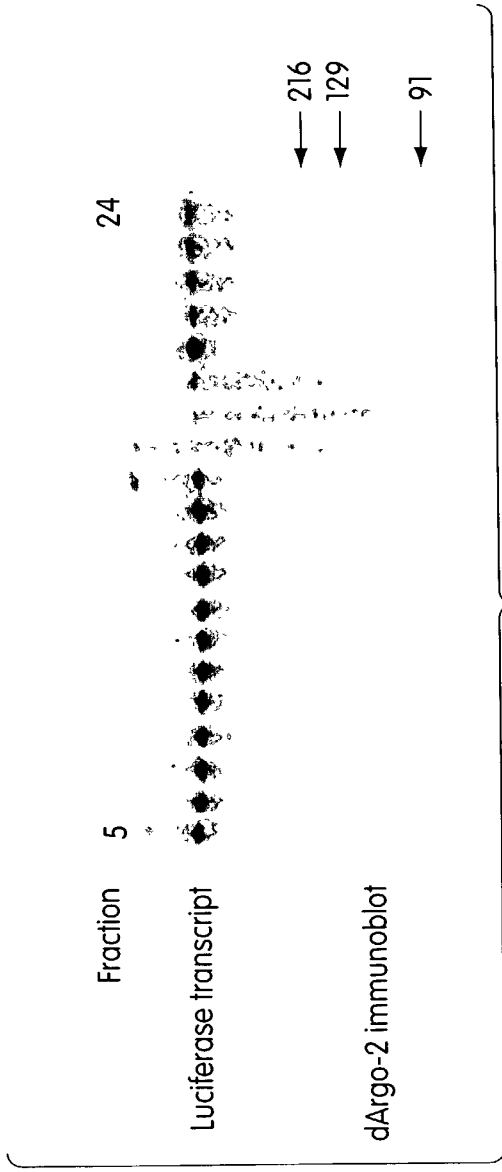

Fig. 12

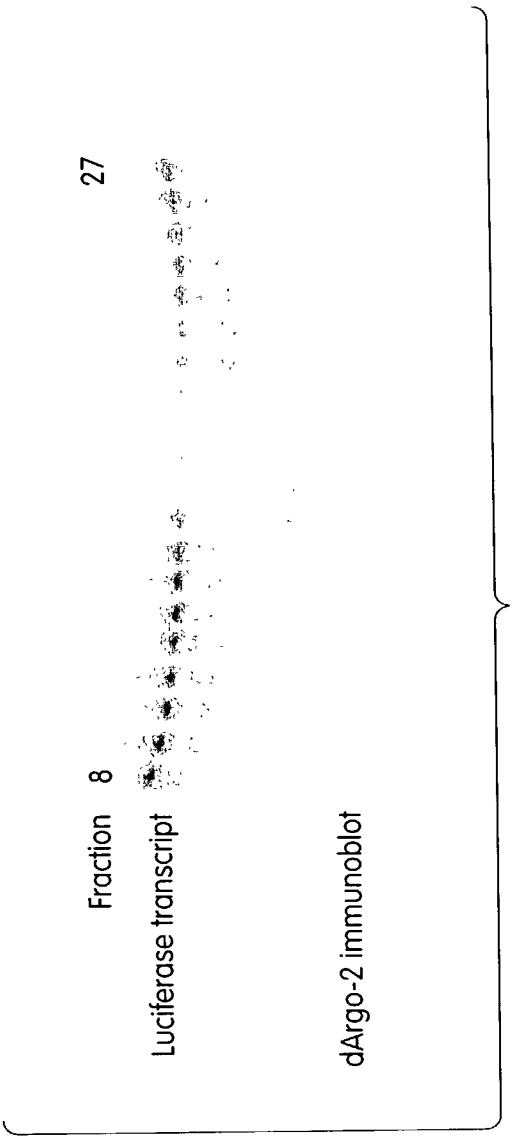

Fig. 13

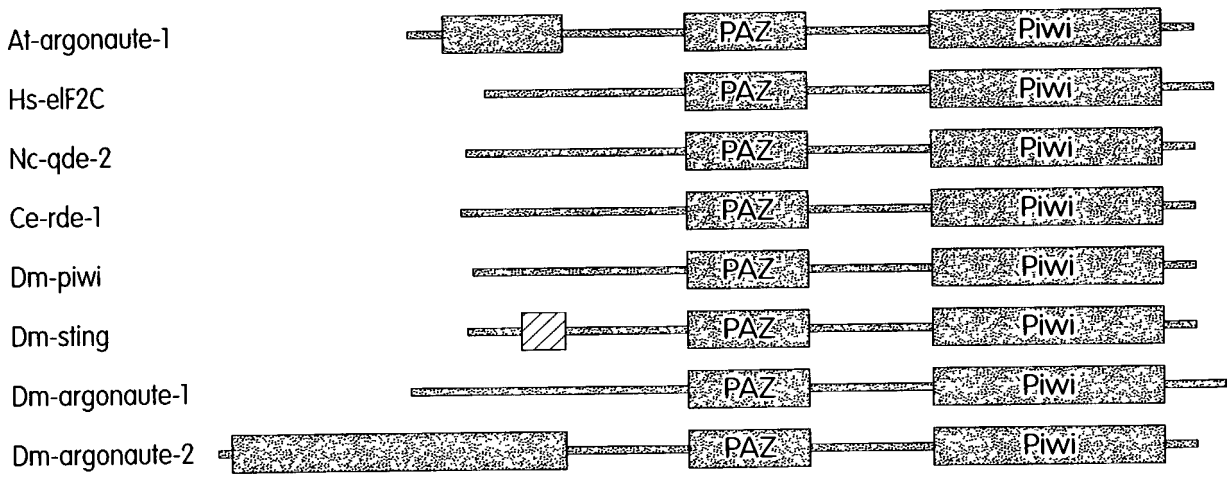

Fig. 14

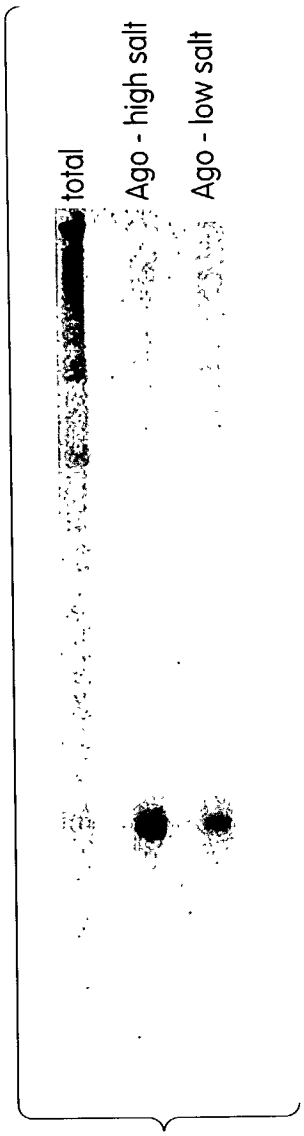

Fig. 15

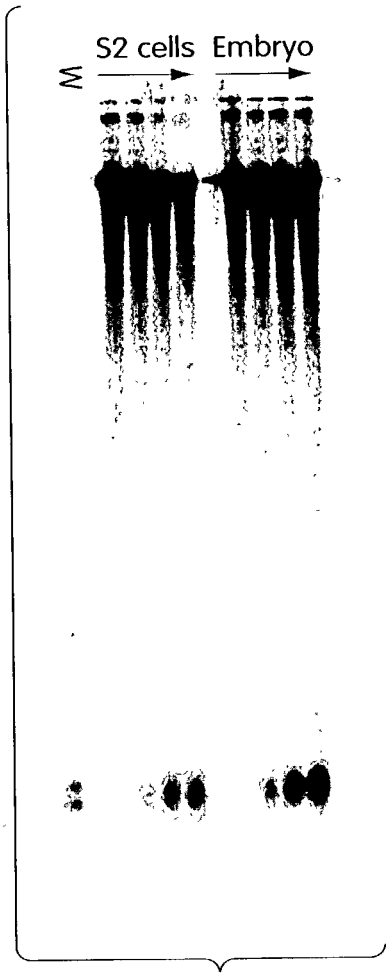

Fig. 16

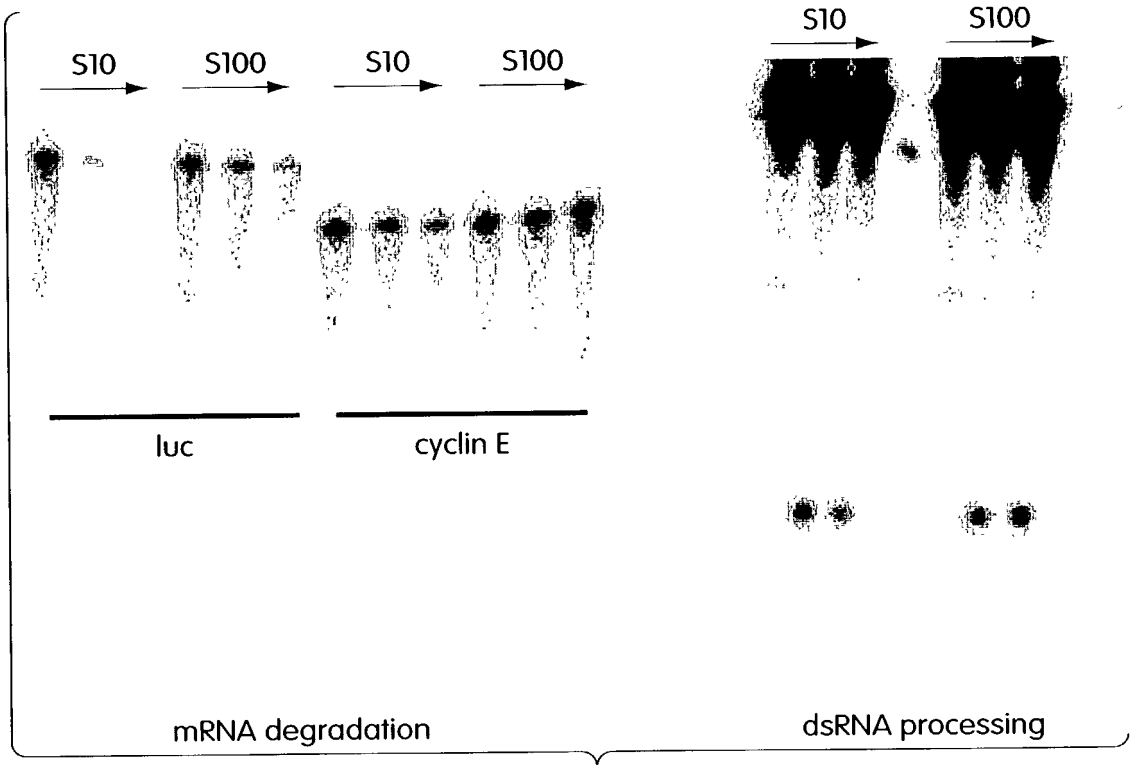

Fig. 17

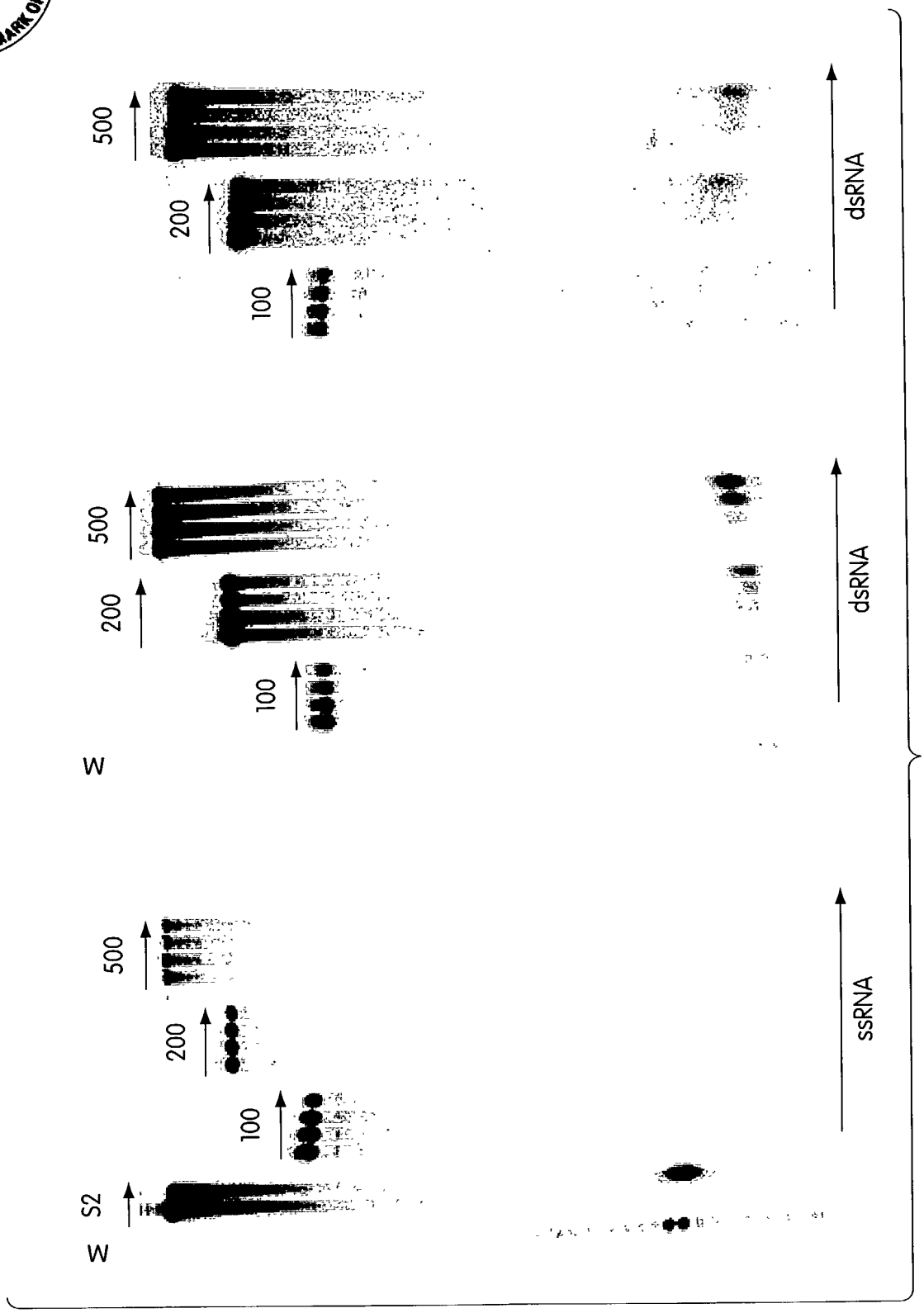

Fig. 18

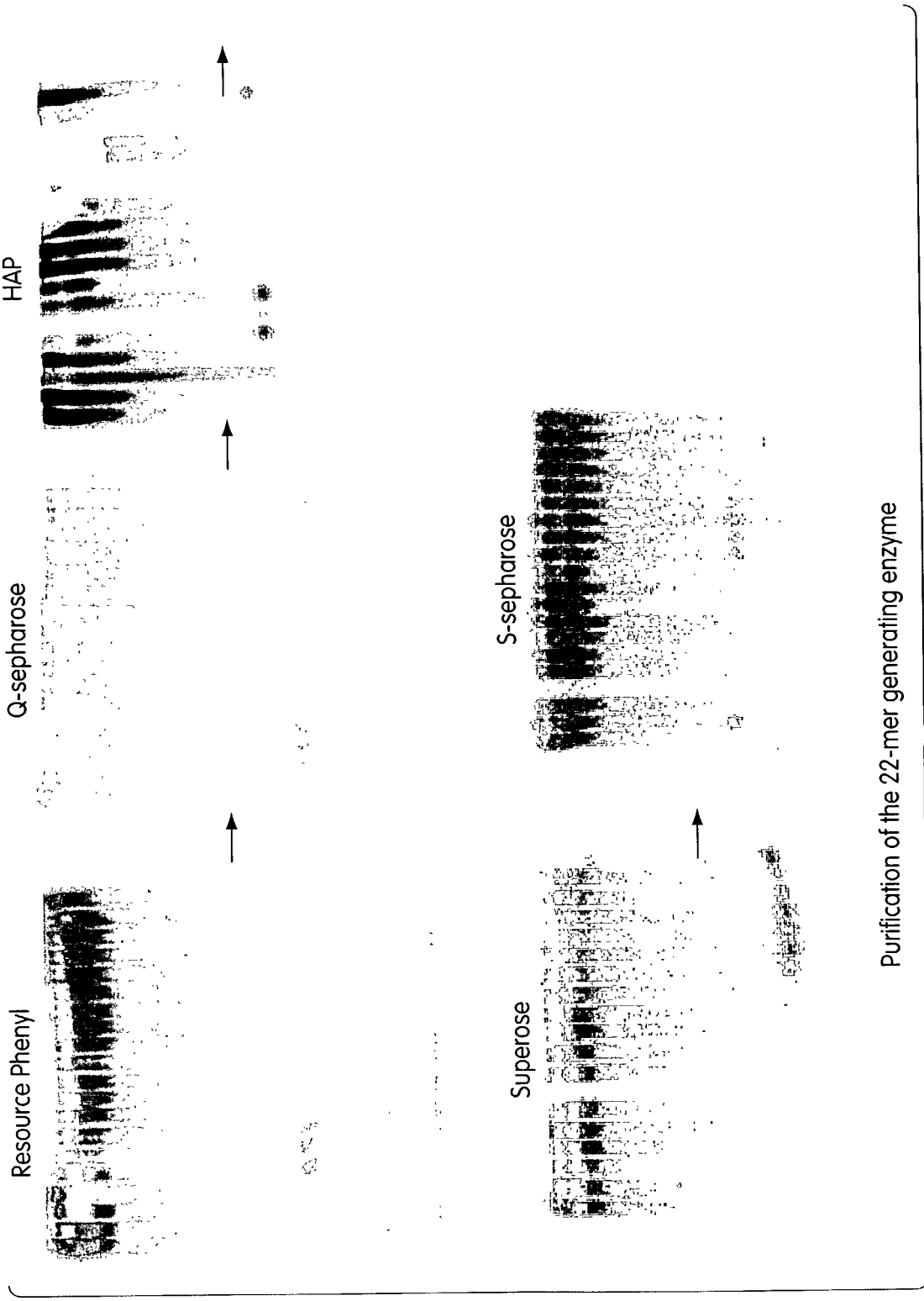

Fig. 19

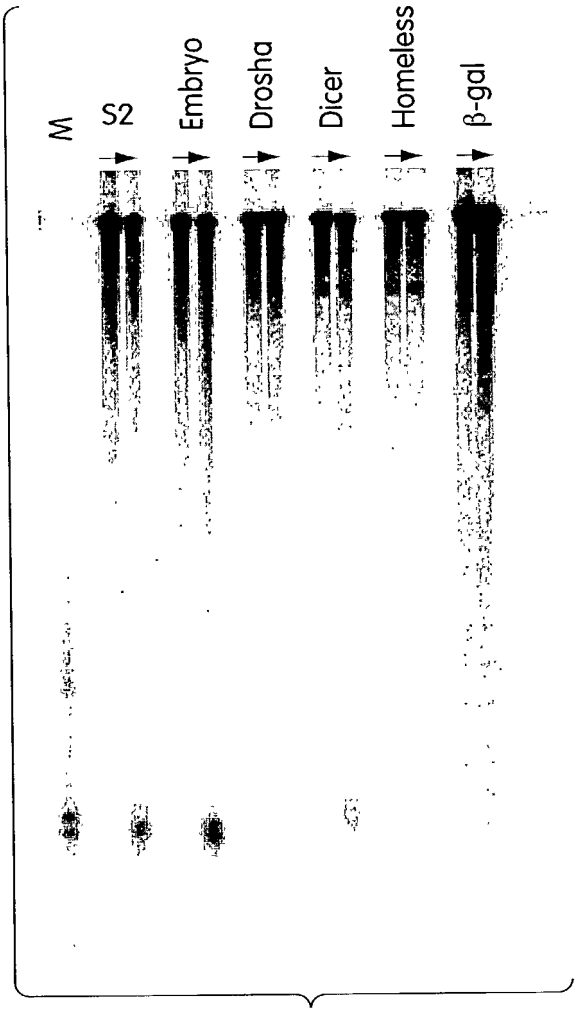

Fig. 20A

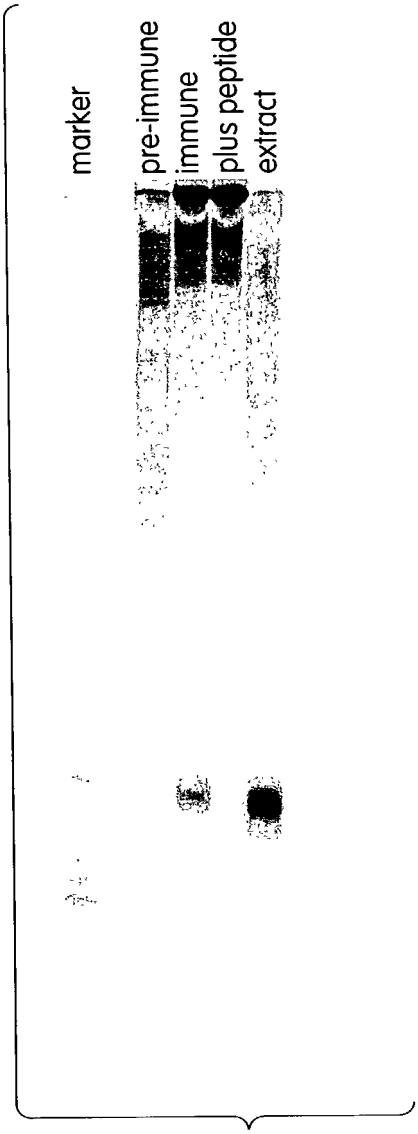

Fig. 20C

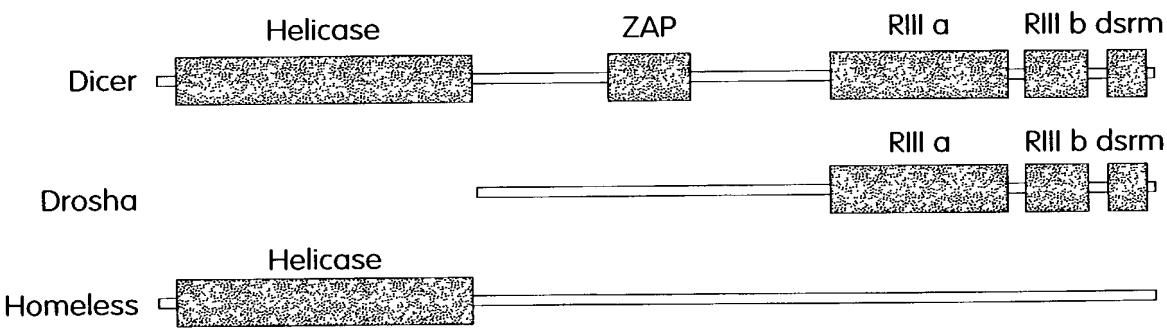

Fig. 20B

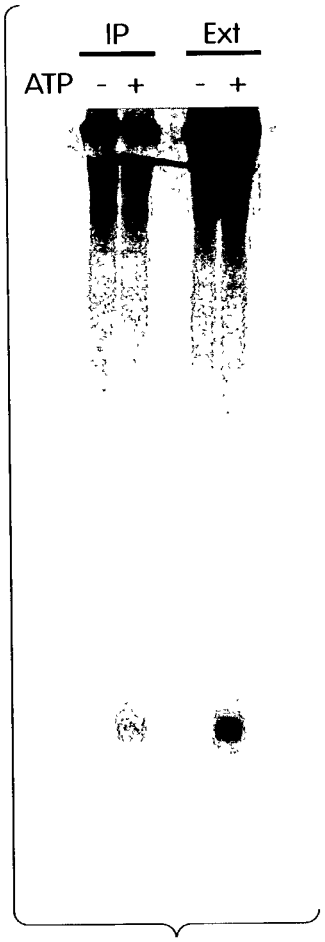

Fig. 21

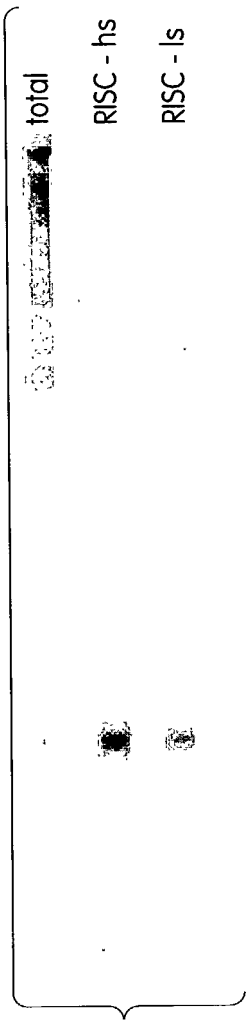

Fig. 22A

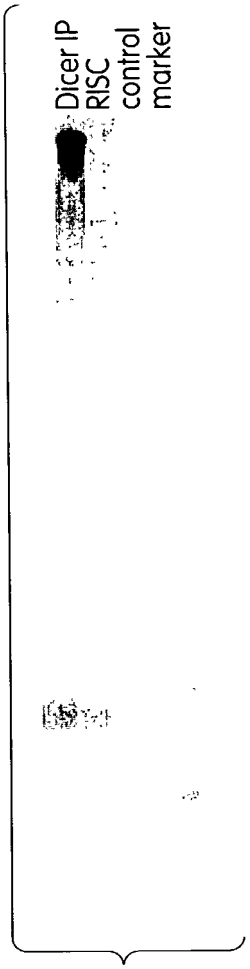

Fig. 22B

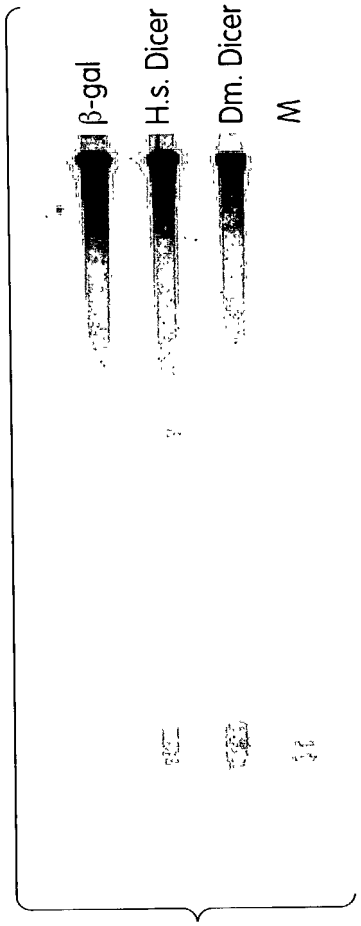

Fig. 23

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DISHKSFPISMPMIEYLERFSLKAK**INNTTNLDYSR**RFLEPFLRGINVVYTPPQSFQS
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Fig. 24

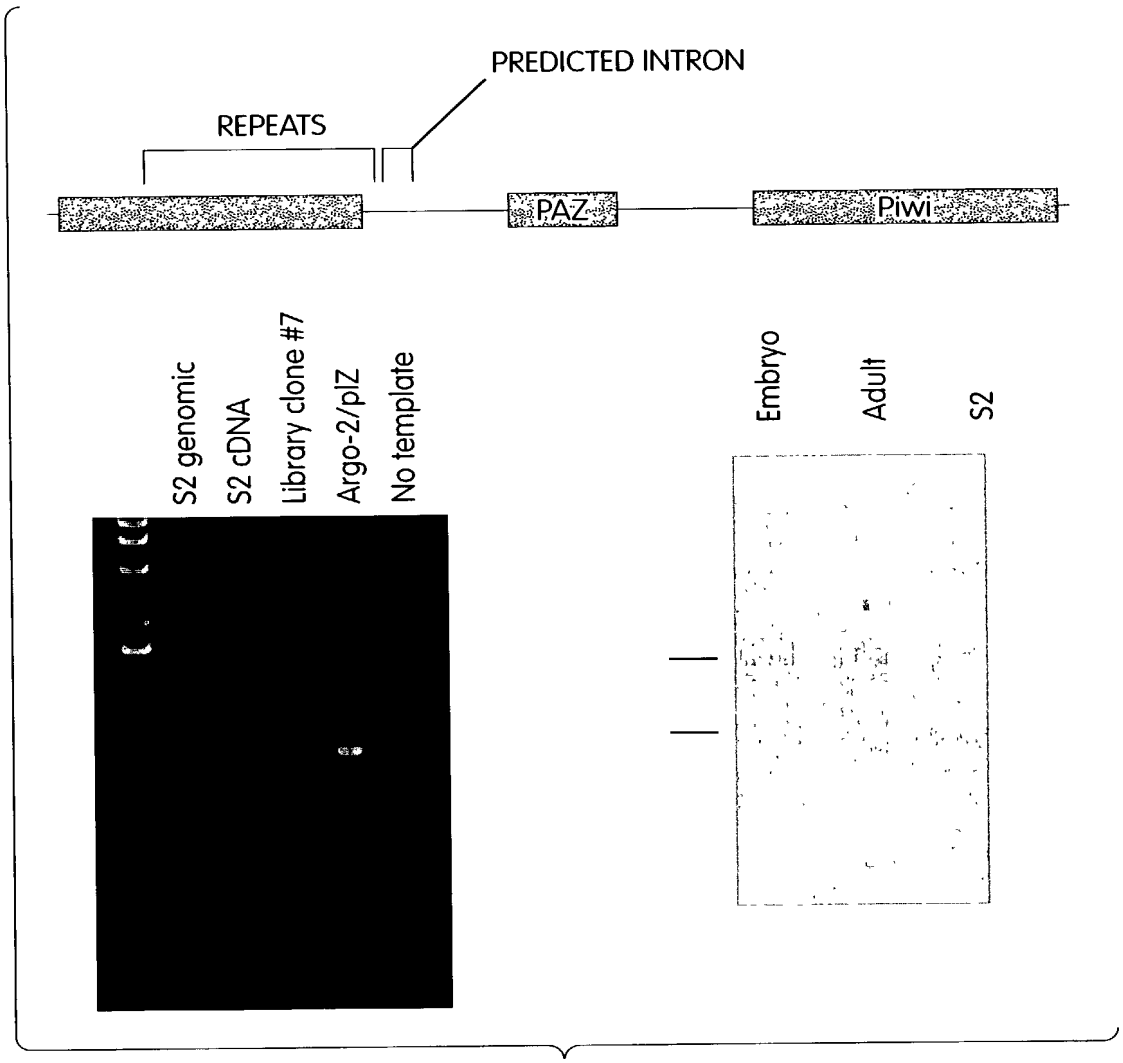


Fig. 25

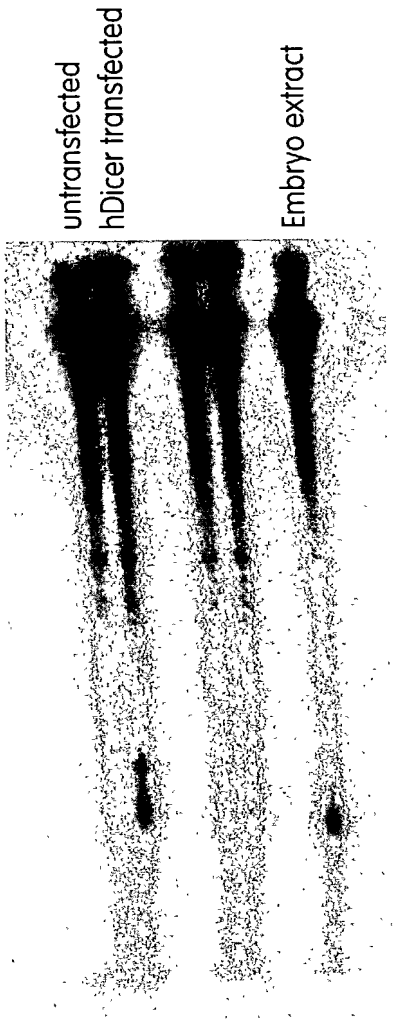
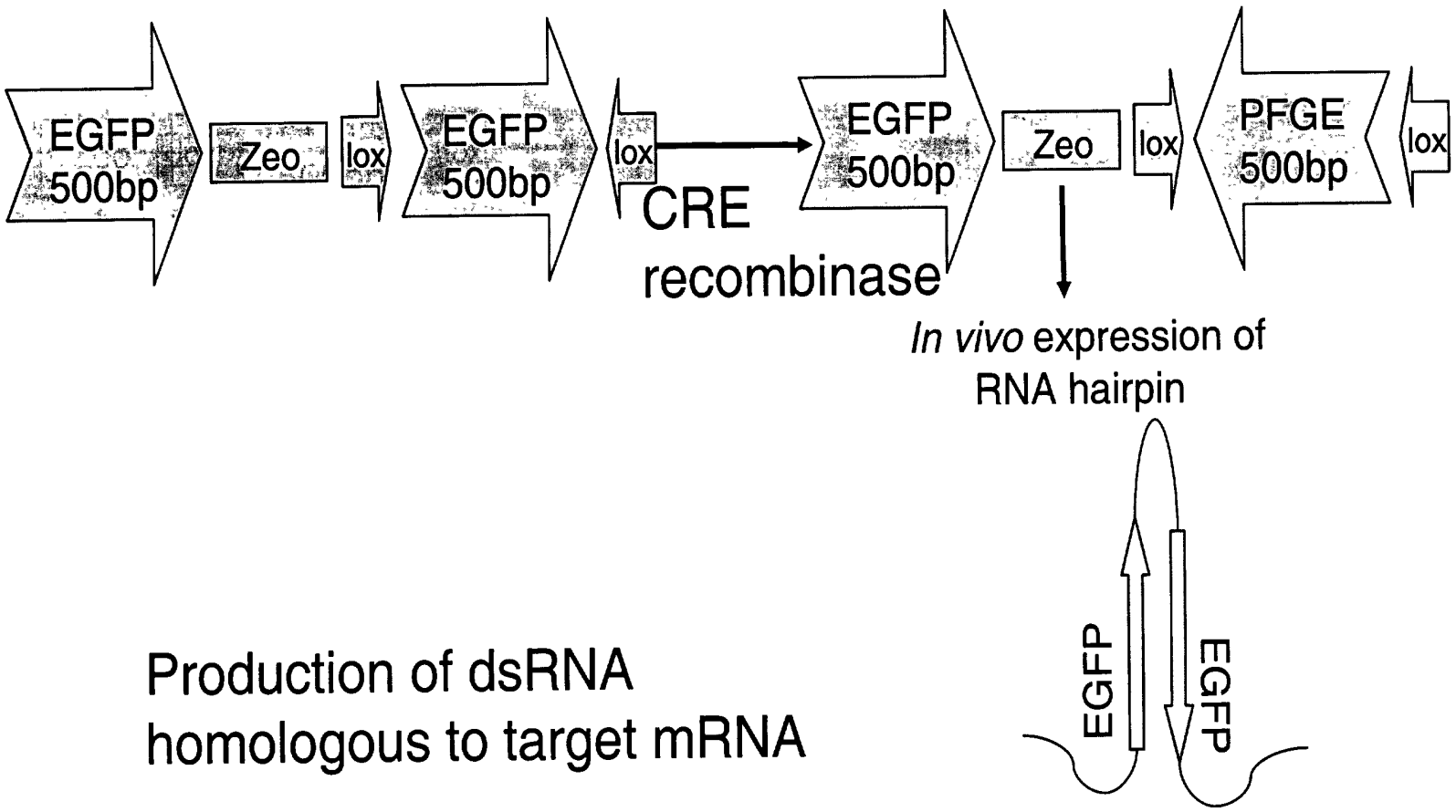


Fig. 26

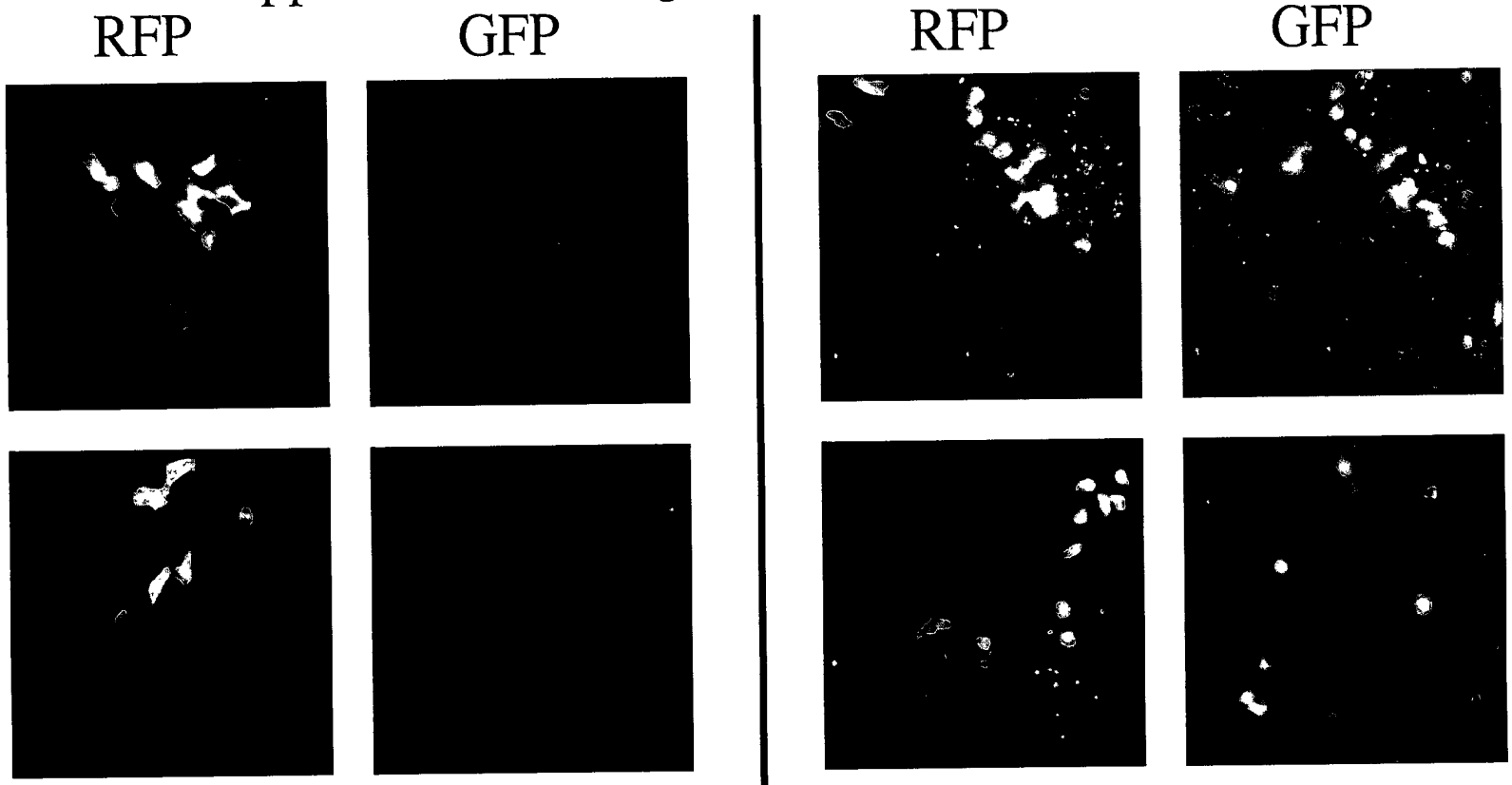
Figure 27
Strategy for stable expression of dsRNA in cultured mammalian cells

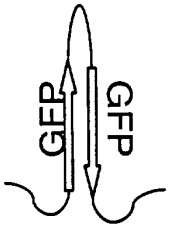


Production of dsRNA homologous to target mRNA

Figure 28

Stable suppression of transgene expression in ammalian cells





Putative P19 clones stably ex-pressing 500mer GFP hairpin

wt P19

Co-transfection with pRFP and pGFP, 42 hrs post-transfection

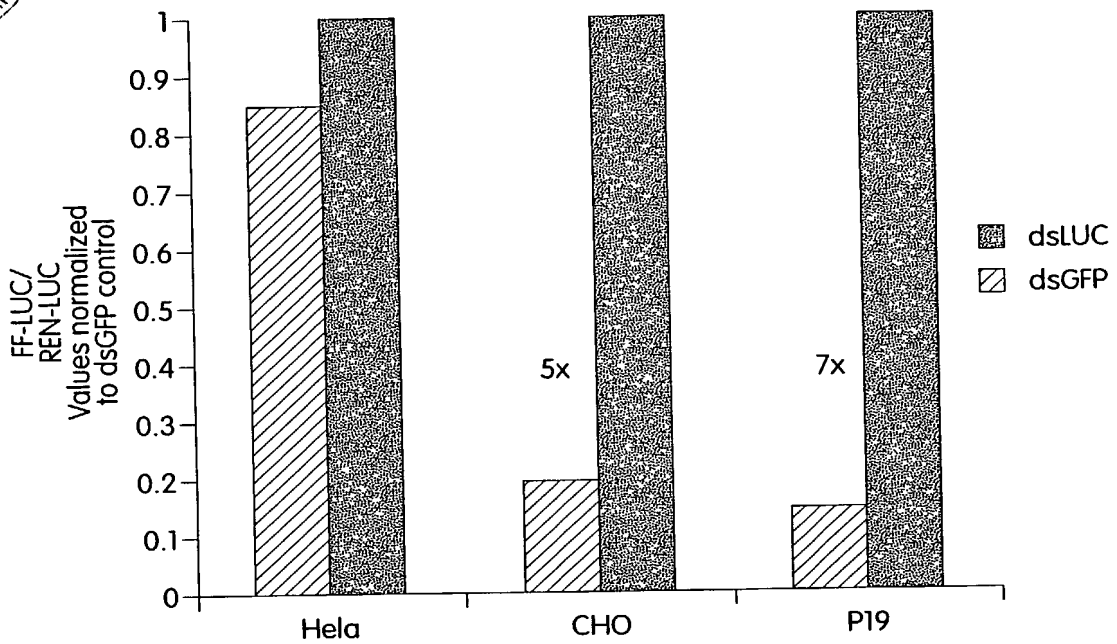


Fig. 29

Figure 30
RNAi in ES cells

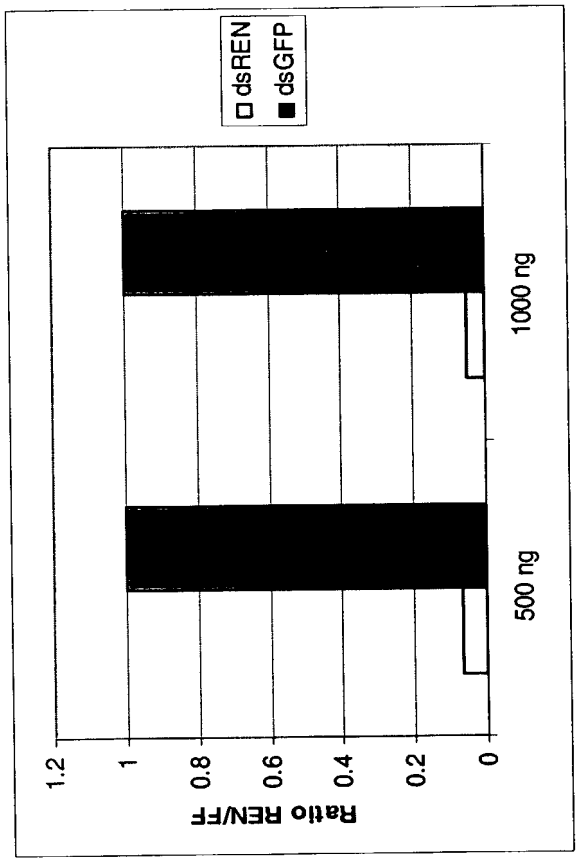
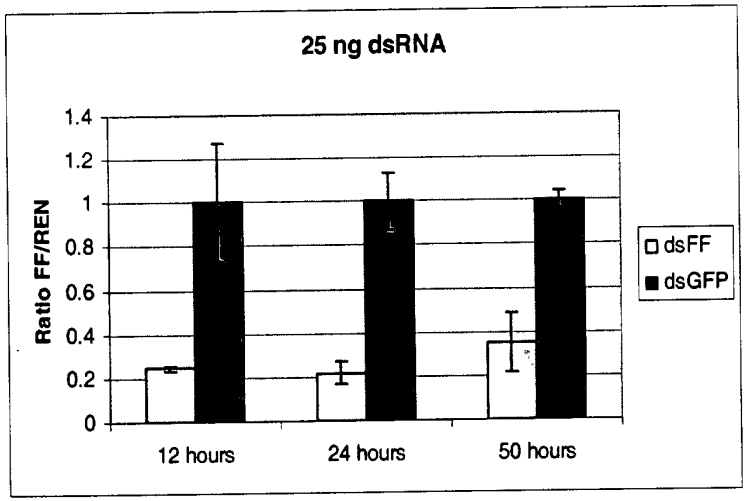


Figure 31
RNAi in mouse embyronic cells (P19)



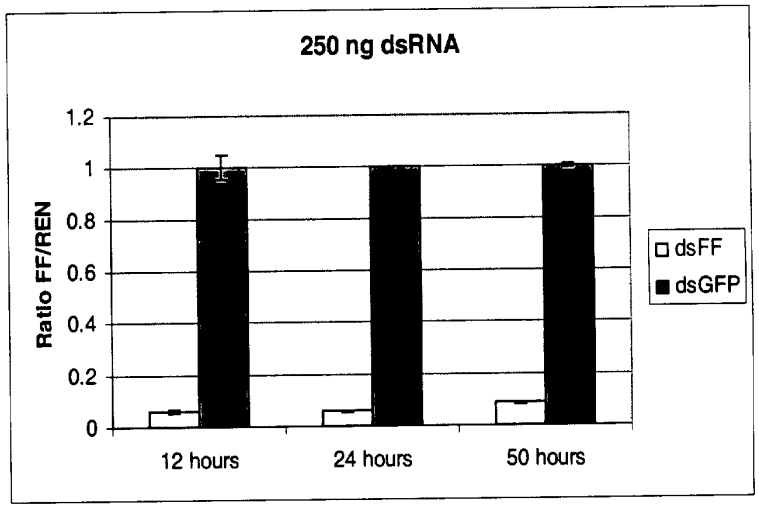


Figure 32
RNAi is post-transcriptional

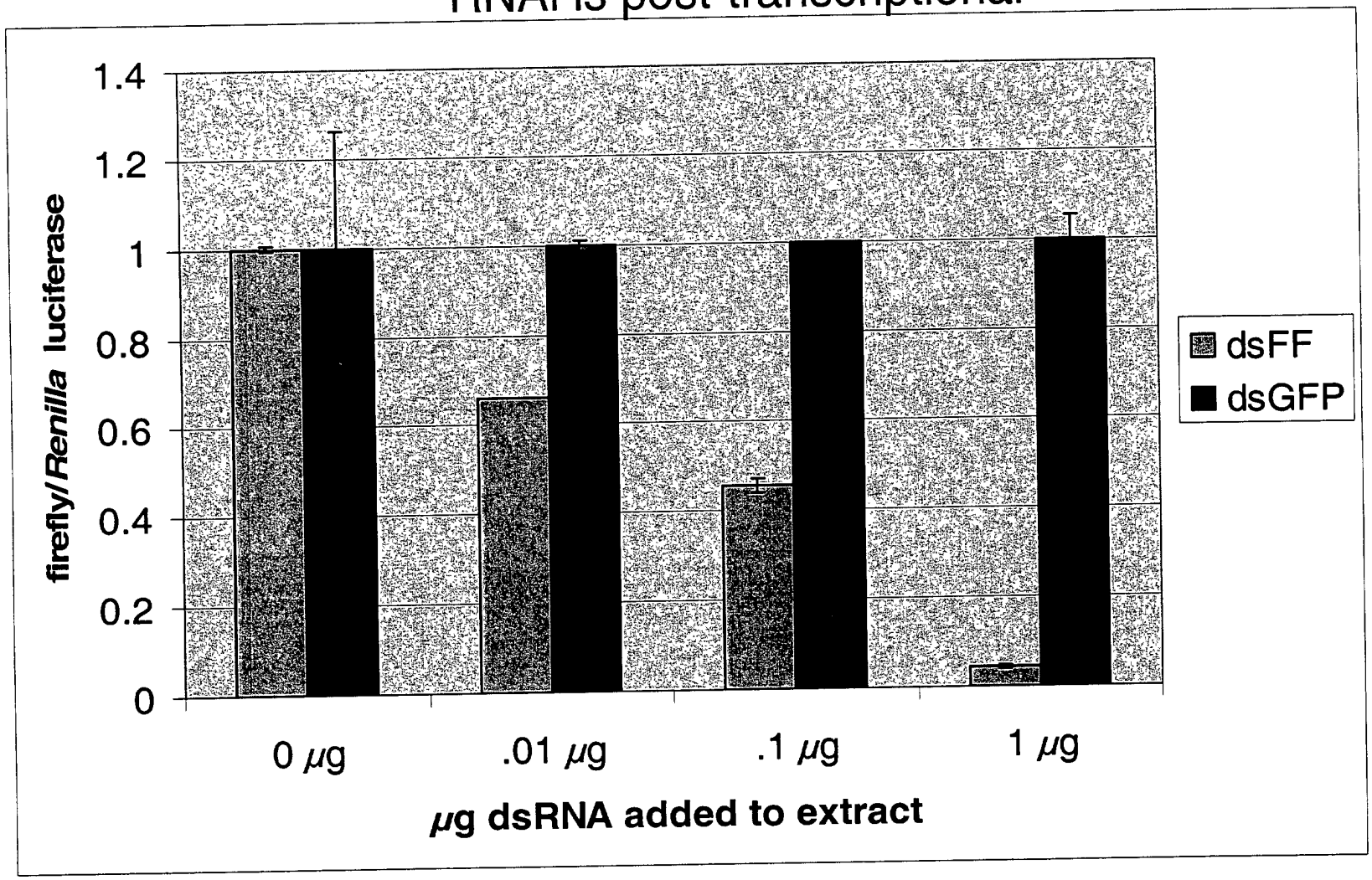
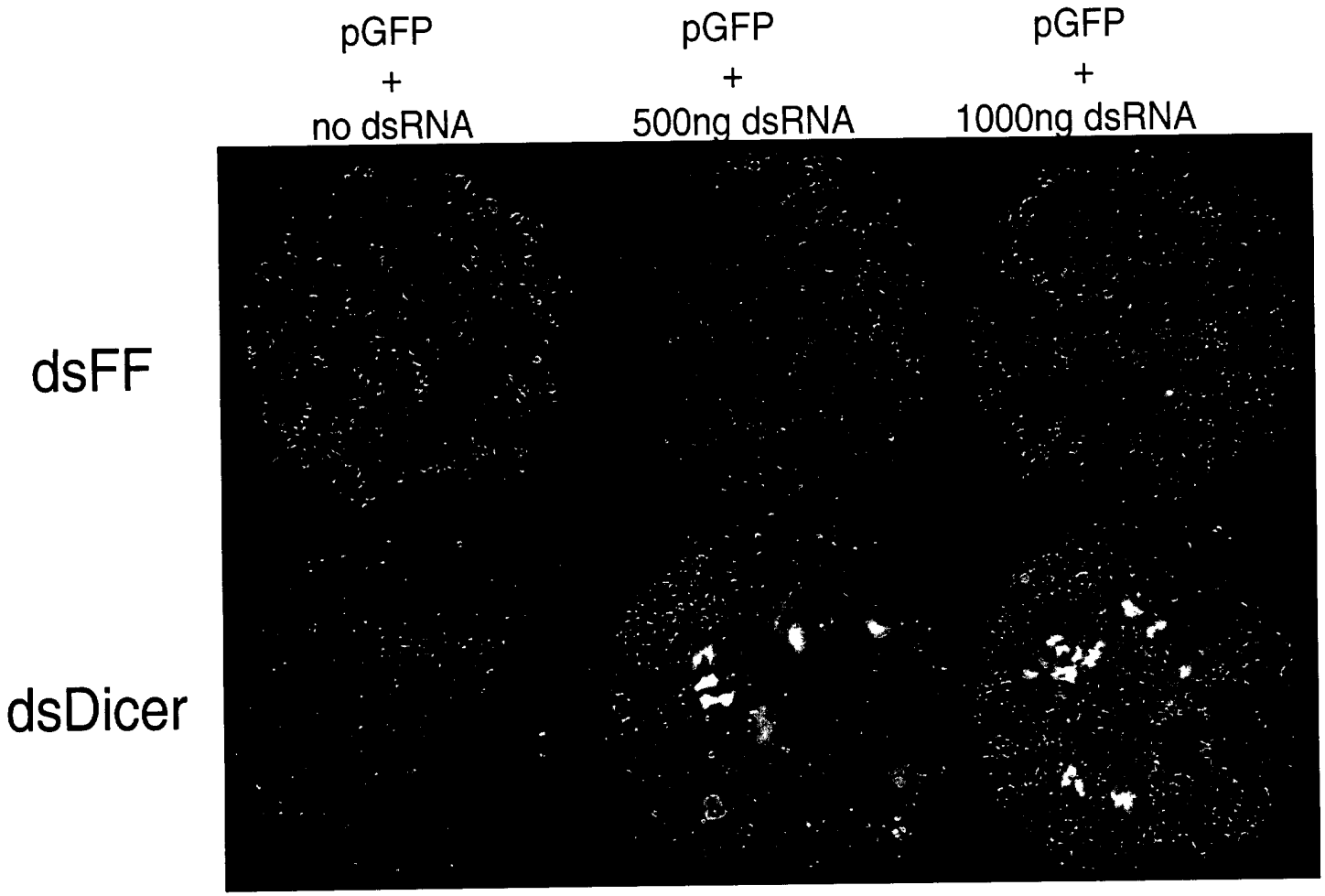
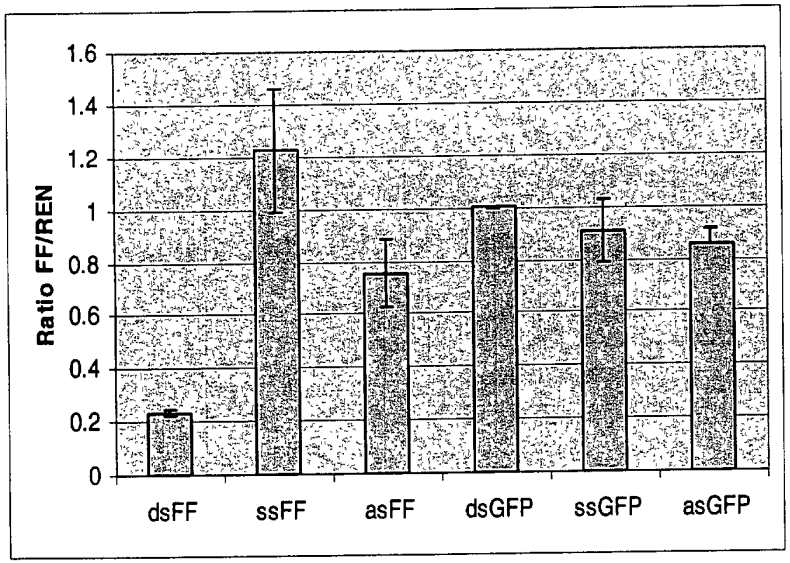


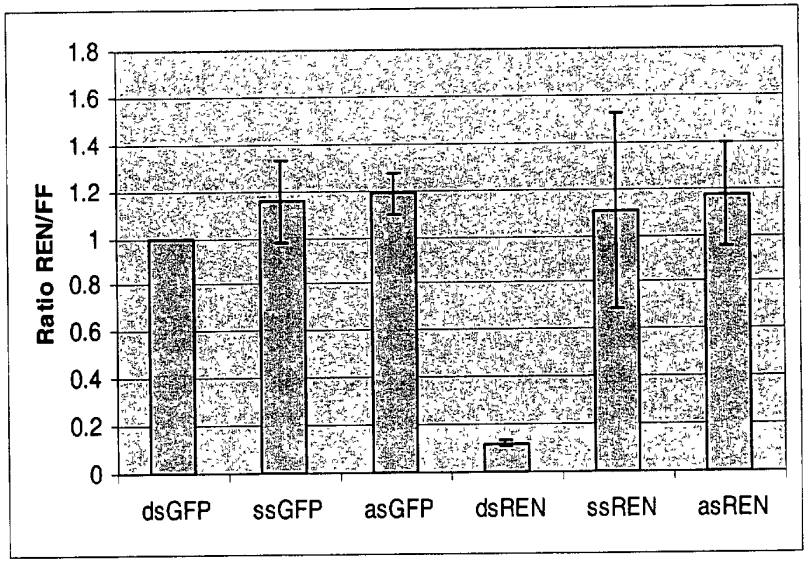
Figure 33



P19 GFP hairpin clone number #10
48hrs post-transfection
Fluorescent microscopy superimposed with bright field

Figure 34
Silencing is specific and requires dsRNA





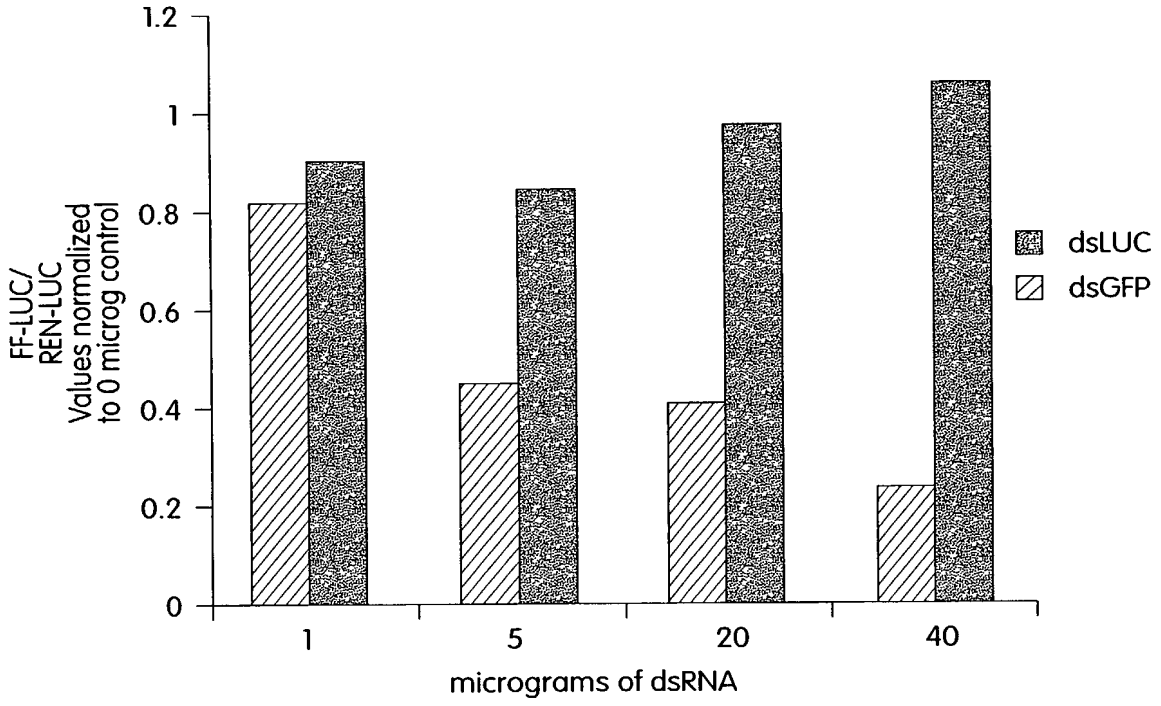
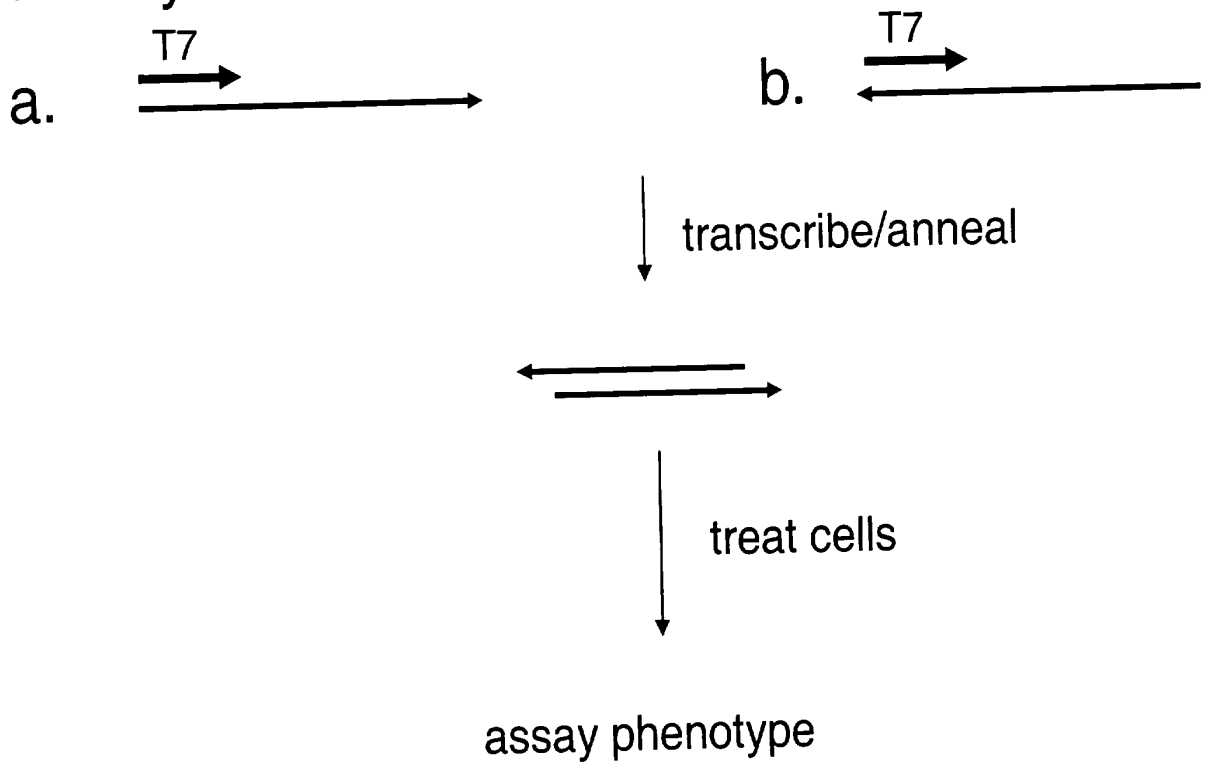


Fig. 35

Figure 36
In vitro synthesis of siRNAs by T7 RNA polymerase

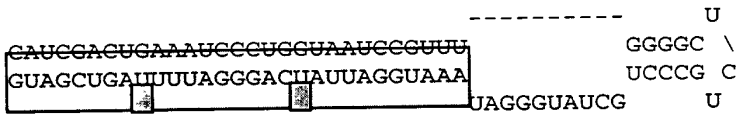


DNA synthesis/RNA transcription
~ $16/siRNA versus ~$400/siRNA for chemical synthesis
Brings large-scale projects within reasonable budget range

Figure 37

Luciferase siRNA

Luciferase Let-7 like



Luciferase simple hairpin

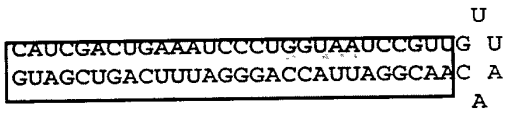


Figure 38
Short Hairpin RNAs in Drosophila S2 cells

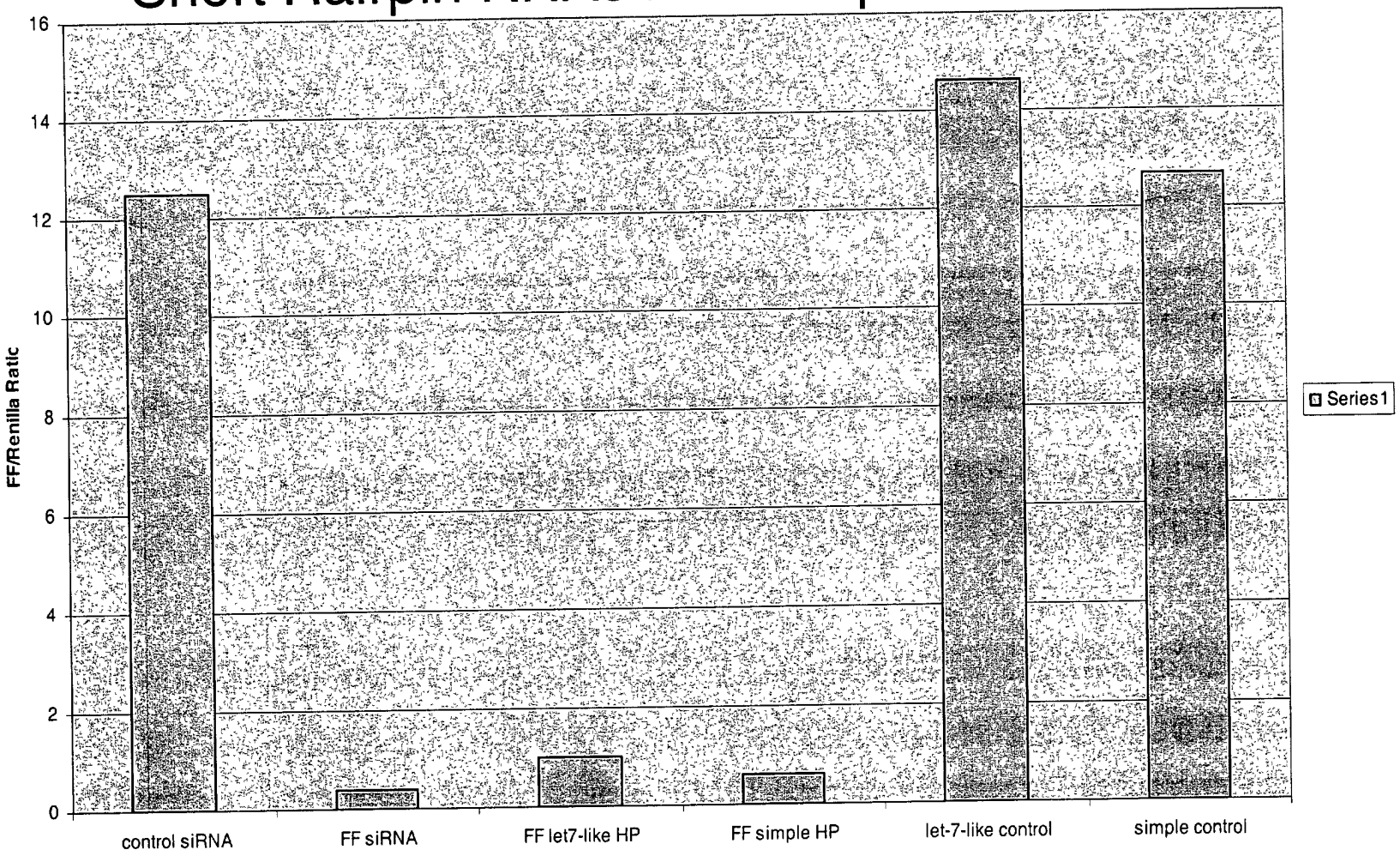


Figure 39
Short Hairpin RNAs in Human 293T cells

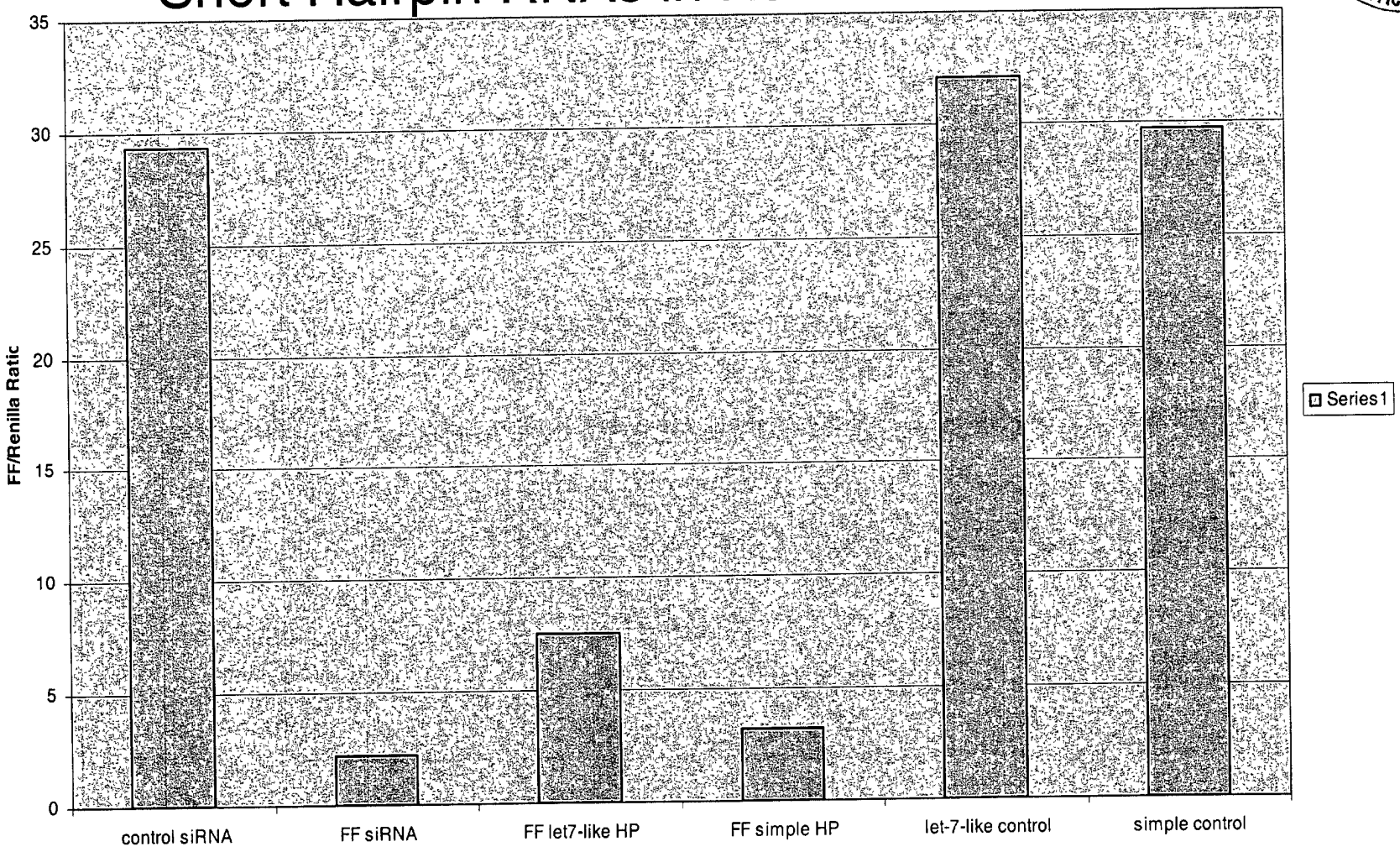


Figure 40
Short Hairpin RNAs in Human HeLa cells

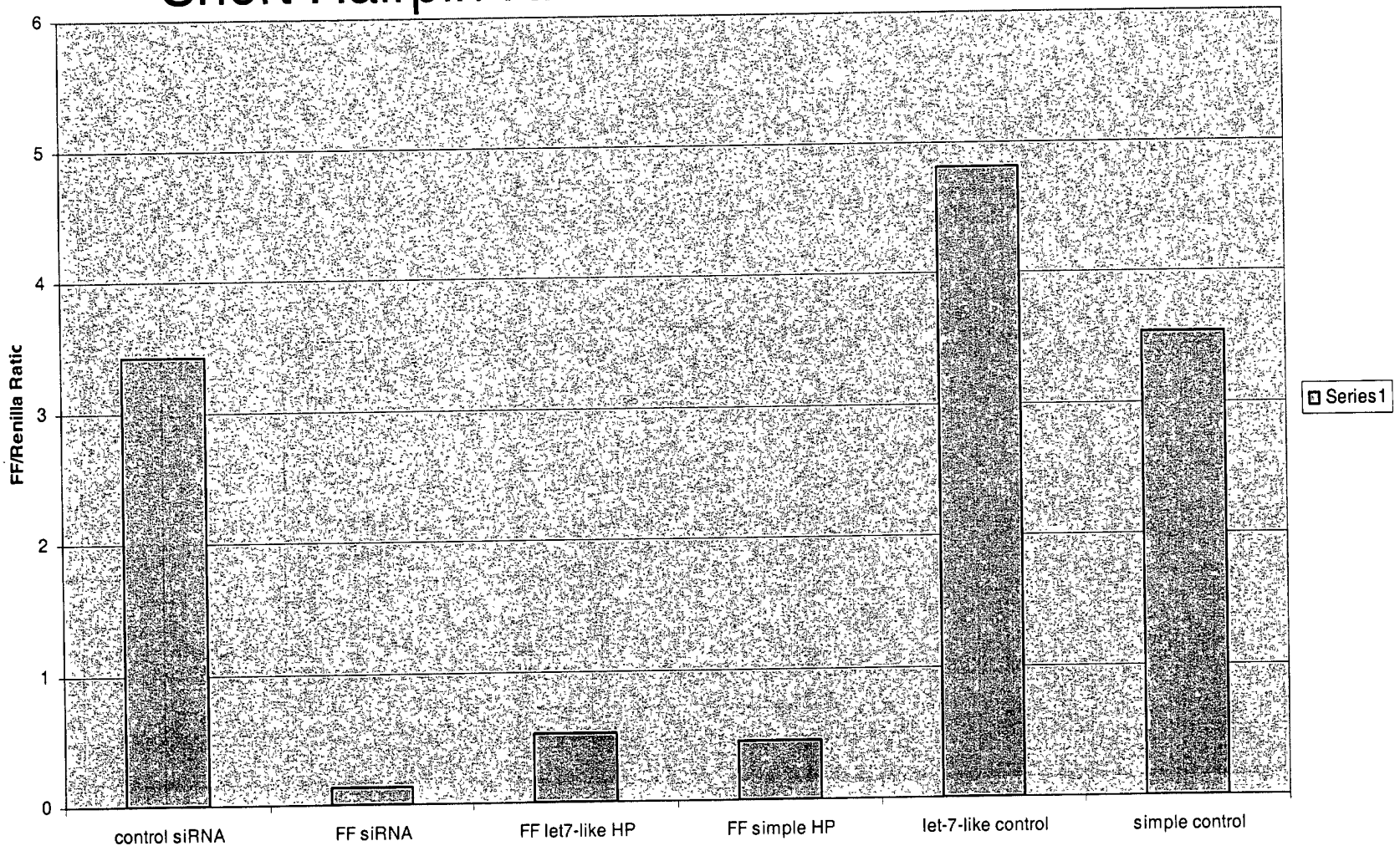


Figure 41
Simultaneous introduction of multiple hairpins does not produce synergy

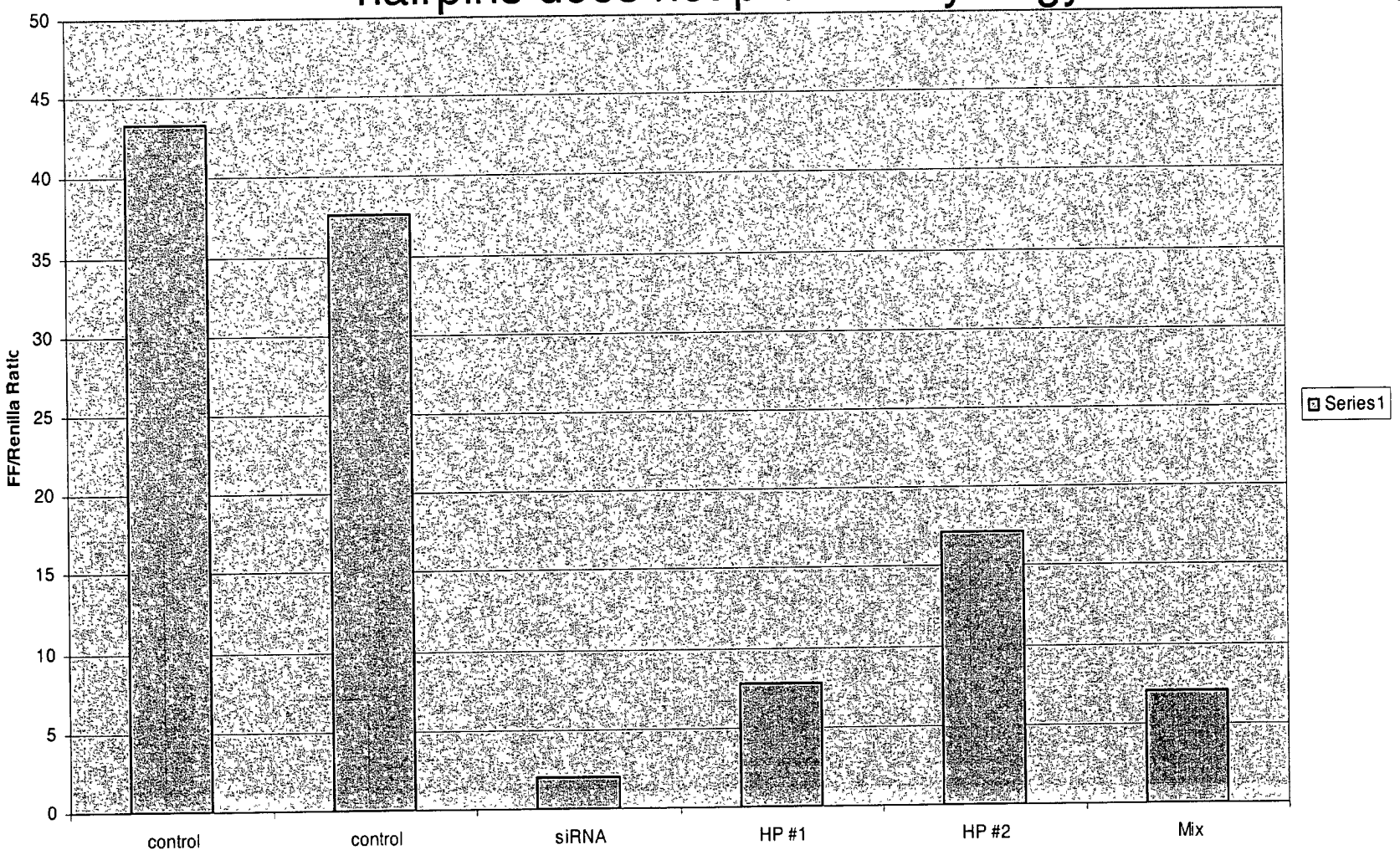


Figure 42

Encoded short hairpins function *in vivo*

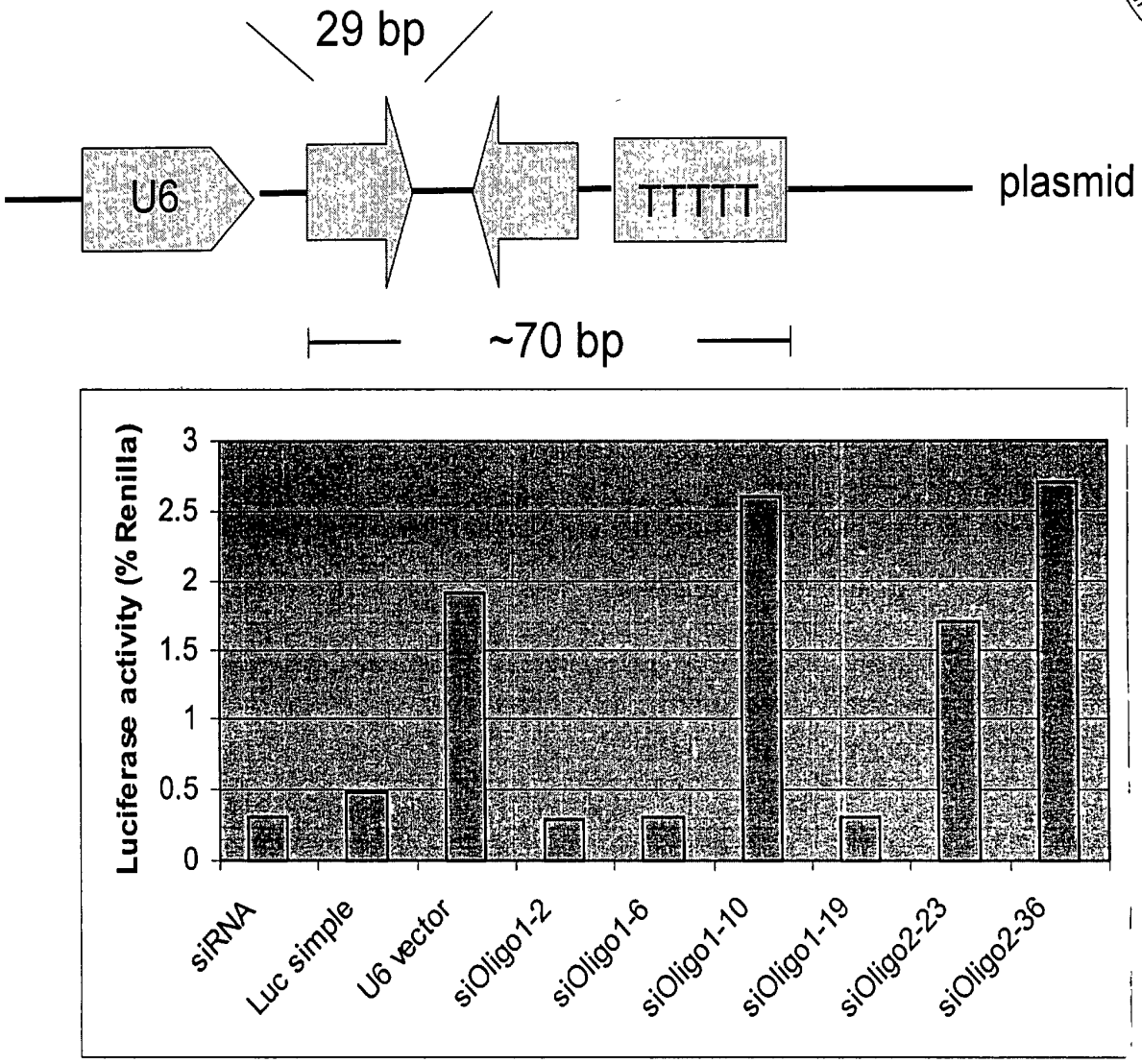
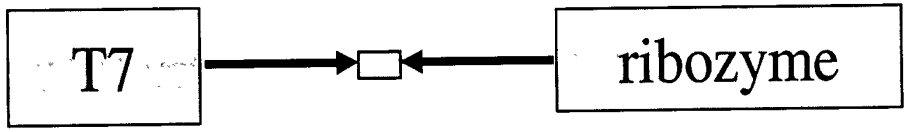
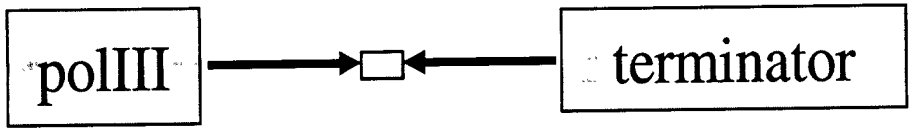


Figure 43

Stable Suppression by short dsRNAs – stable expression strategies

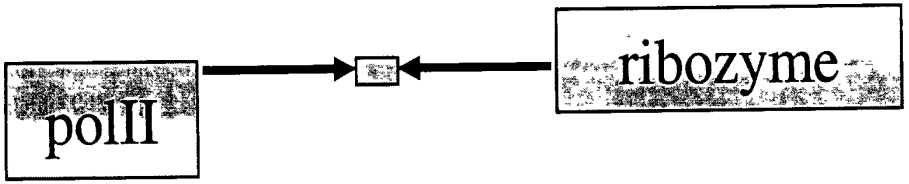


T7 gives site-specific initiation. 3' end formation
Achieved with ribozyme (e.g. hepatitis delta virus ribozyme).



polIII gives site-specific initiation.
Example promoters – U6 snRNA, H1 RNA, SRP RNAs (7SL)
3' end formation
Achieved with native terminator (e.g. TTTTT). Leaves the last TT, so that could be used to pair to transcript.

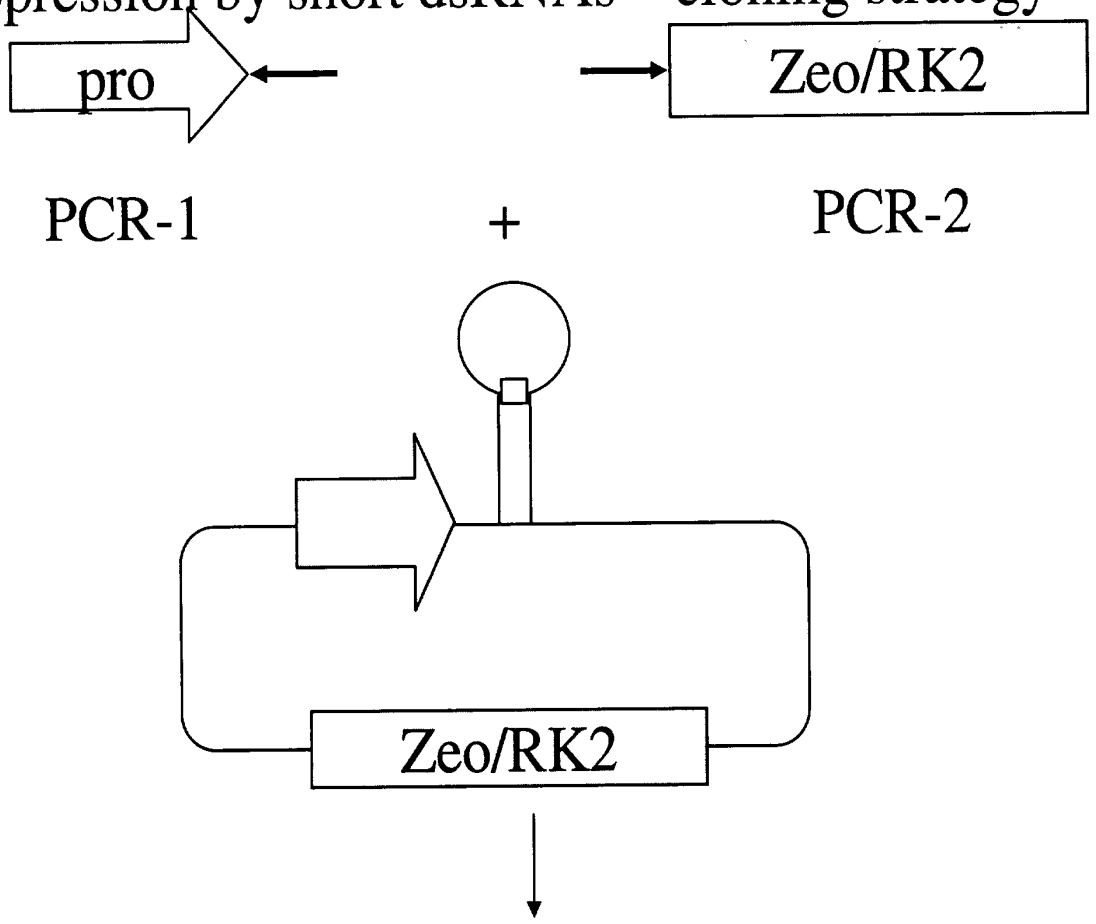
Could also use VA1, tRNA etc but would have to couple with Ribozyme since those promoters need also internal elements.



polII gives site-specific initiation. Example promoters
Would be U1 snRNA promoters, CMV etc...
3' end formationachieved with ribozyme
(e.g. hepatitis delta virus ribozyme).

Figure 44

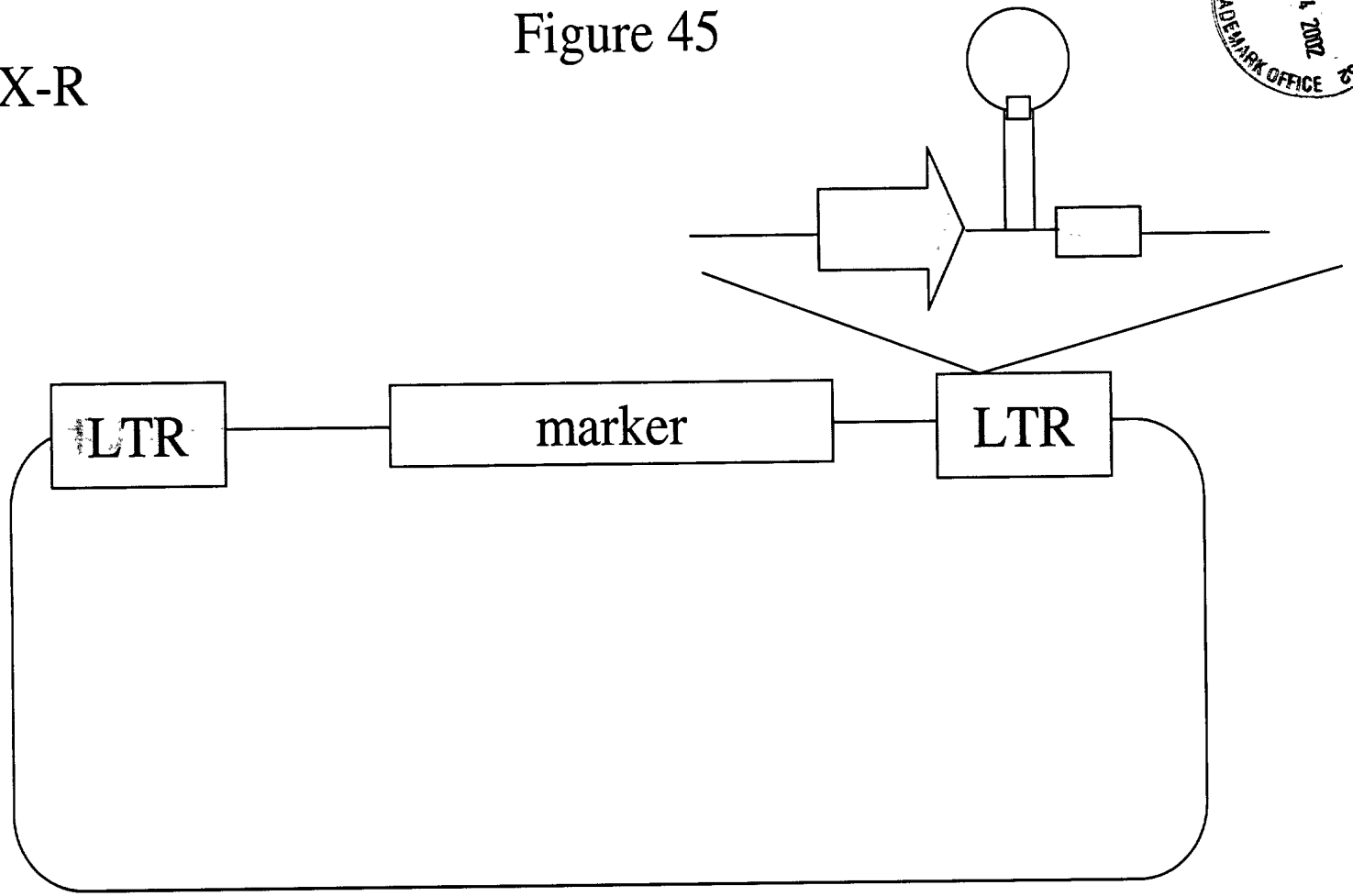
Stable Suppression by short dsRNAs – cloning strategy



Automatic subcloning into vector of choice

Figure 45

MaRX-R



Stable suppression by expressed RNAi

Figure 46

Early Passage PKR -/- MEFs: dual luciferase assay with long dsRNA (~500nt)

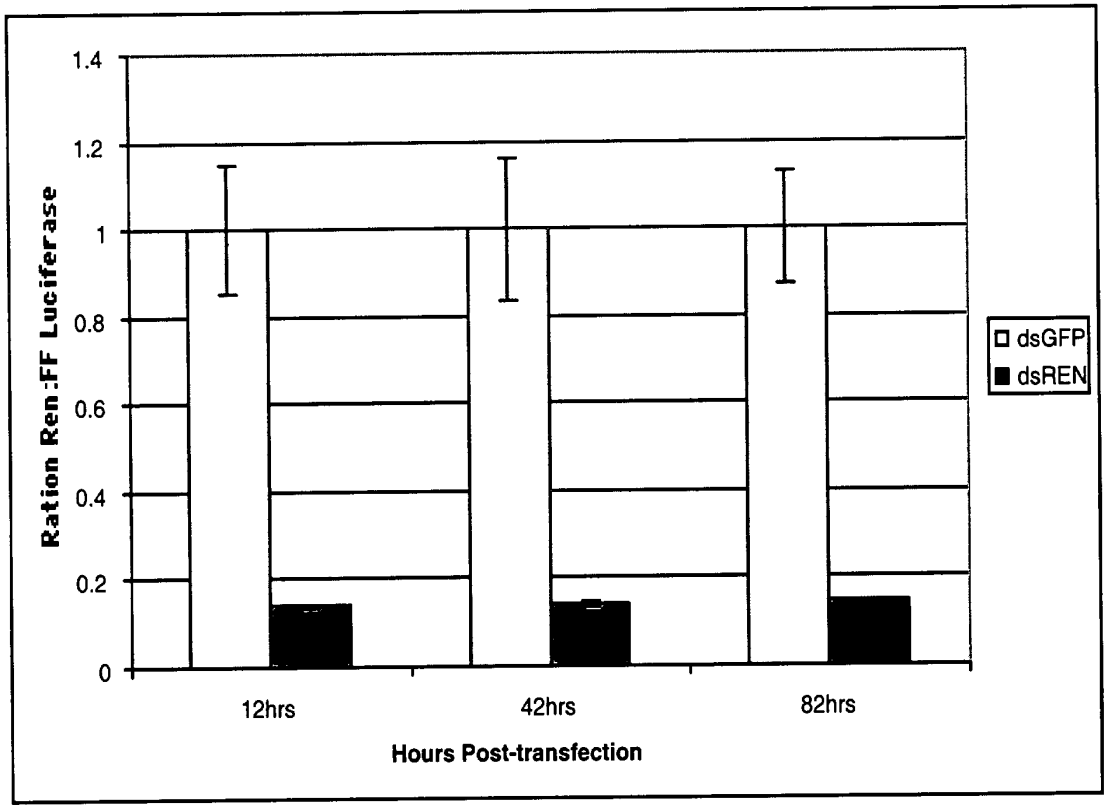


Figure 47

Mouse Tyrosinase Promoter